

# Aquatic Life Water Quality Criteria For Molybdenum



Prepared for Nevada Division of Environmental Protection Bureau of Water Quality Planning



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# **Executive Summary**

Molybdenum is a naturally occurring metal element that typically occurs in combination with other elements in a variety of forms. In general, this element is geochemically mobile and tends to enter water under normal conditions. It is also an essential micronutrient found in all plant, animal, and human tissues. Molybdenum is actively mined and primarily used commercially as an alloying agent to strengthen and harden steel and increase wear and corrosion resistance in resulting alloys. Global and domestic demand for molybdenum has increased over the past several years, as has the price per kilogram of molybdenum. Currently, there are at least two mines producing molybdenum in Nevada and at least one molybdenum mine is currently being planned. As such, it is likely that water quality criteria and standards for molybdenum will become increasingly useful for evaluating potential molybdenum effects as well as permitting mine discharges in Nevada.

The U.S. Environmental Protection Agency (EPA) has not developed a national water quality criterion for molybdenum for protection of aquatic life. This is probably at least partly due to the relatively low toxicity and rare occurrence of molybdenum in most areas of the U.S. Typically, the dietary effects of excessive molybdenum on livestock are of much greater concern than are toxic effects to aquatic life. The current State of Nevada (2006 NAC 445A.144) molybdenum water quality standard (WQS) for protection of the aquatic life beneficial use is 19  $\mu$ g/l, and was adopted based on recommendations contained in a report prepared by the California State Water Resources Control Board for the regulation of agricultural drainage to the San Joaquin River. The 19  $\mu$ g/l standard is strongly biased by the low ambient background concentration, is not toxicologically-based, and was not derived using methods consistent with those recommended by EPA.

The objectives of this project were to develop appropriate molybdenum water quality criteria for aquatic life protection for the State of Nevada that are scientifically defensible, meet USEPA protocols for deriving criteria, and are based on available toxicological data and supporting information.

The scientific literature regarding molybdenum toxicity data was reviewed, including all relevant laboratory toxicity data reported over the past 35 years. Assembled data were screened for use in criteria development based on EPA's checklist and based on the occurrence of the test species in Nevada. Following screening, acute toxicity data were available for 17 species in 13 genera and chronic data were available for five species in five genera. Sufficient acute toxicity data were available to satisfy EPA criteria development methodology, however there were too few chronic data to develop a chronic criterion independent of the acute criterion. Sufficient data were available to calculate an acute-to-chronic ratio (ACR), as recommended by EPA, which was used to calculate a chronic criterion based on relationships established between the acute and chronic toxicity of molybdenum to four different species.

Based on this work and using EPA's methodology, an acute molybdenum criterion of 6.16 mg/L and a chronic molybdenum criterion of 1.65 mg/L (both as total molybdenum) were calculated. These criteria are substantially lower than concentrations of molybdenum determined to have no effect on either natural benthic macroinvertebrate communities or *in situ* test organisms placed in

streams receiving mine discharges. Finally, both the acute and chronic criteria values are much lower than concentrations determined to have no effects on rainbow trout after a year of constant exposure.

The criteria developed in this study were calculated based upon all available, suitable molybdenum data in the scientific literature, appropriate EPA methodology, and should be considered protective of aquatic life in Nevada. Therefore, these criteria are appropriate for adoption as water quality standards for the State of Nevada.

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#### 1.0 Introduction

Molybdenum is a naturally occurring metal element that typically occurs in combination with other elements in a variety of forms. In general, this element is geochemically mobile and tends to enter water under normal conditions. In solutions with a pH of greater than 5, the molybdate ion is the dominant form of molybdenum, although many other forms and polymers exist depending on the pH and other chemical characteristics of the system (Hem 1985). Of the various mineral forms that naturally occur, molybdenite (MoS<sub>2</sub>) is the only mineral with significant commercial value. Molybdenum is actively mined and primarily used commercially as an alloying agent to strengthen and harden steel and increase wear and corrosion resistance in resulting alloys (IMOA 1998, USGS 2007).

Global and domestic demand for molybdenum has increased over the past several years, as has the price per kilogram of molybdenum (USGS 2007). The U.S. Geological Survey (2007) estimates that the United States has approximately 5.4 million tons of identified molybdenum resources, while the rest of the world has approximately 13 million tons. Currently, there are at least two mines producing molybdenum in Nevada (the Ashdown Mine near Denio and the Robinson Mine near Ely) and at least one molybdenum mine (the Mt. Hope Mine) is currently being planned. As such, it is likely that water quality criteria and standards for molybdenum will become increasingly useful for evaluating potential molybdenum effects as well as permitting mine discharges in Nevada.

#### 1.1 Ambient Molybdenum Concentrations in Nevada

Ambient monitoring data provided by NDEP (Table 1.1) indicate that molybdenum has been observed in most, if not all, major river basins and regions of Nevada. Maximum recorded values in most river basins are less than 100  $\mu$ g/L, but have been measured as high as 210  $\mu$ g/L in the Carson River basin and 470  $\mu$ g/L in the Walker River Basin. Of all basins, only the Walker River has been observed to contain mean molybdenum values in excess of 11  $\mu$ g/L (188  $\mu$ g/L) suggesting that elevated concentrations of molybdenum are more common in this basin. The median molybdenum value observed in most basins is 5  $\mu$ g/L, which was likely the detection limit of the method used to analyze for molybdenum in collected samples. These results indicate that the plurality (if not majority) of samples collected in these systems do not contain molybdenum concentrations in excess of the detection level.

#### 1.2 Biological Necessity

Molybdenum is an essential micronutrient found in all plant, animal, and human tissues (Eisler 1989). As discussed below, the U.S. Environmental Protection Agency (EPA) has not developed a national water quality criterion for molybdenum for protection of aquatic life. This is probably at least partly due to the relatively low toxicity and rare occurrence of molybdenum in most areas of the U.S. Typically, the dietary effects of excessive molybdenum on livestock are of much greater concern that the toxic effects to aquatic life. Concentrations of molybdenum in diets of sheep and cattle of 10 mg/kg can cause disease (Opresko 1993). As such, molybdenum limitations and standards have typically focused more on waters used for irrigation and land disposal of wastes containing molybdenum (e.g., sludge for sewage treatment plants).

Nevada River Basin or		Molybdenum µg/L			Sample
Region	Minimum	Maximum	Mean	Median	Size
01 - Northwest Region	5	5	5	5	25
02 - Black Rock Desert Region	0.25	40	7.90	5	48
03 - Snake River Basin	0.34	10	5.00	5	202
04 - Humboldt River Basin	0.33	42	6.48	5	164
06 - Truckee River Basin	5	22	5.10	5	267
08 - Carson River Basin	5	210	10.86	5	150
09 - Walker River Basin	5	470	188.07	10	153
10 - Central Region	0.25	15	4.89	5	163
11 - Great Salt Lake Basin	5	5	5	5	1
13 - Colorado River Basin	5	25	8.74	5	50

**Table. 1.1.** Summary of ambient molybdenum concentrations ( $\mu$ g/L) measured by Nevada Division of Environmental Protection in various river basins and regions of Nevada.

#### 1.3 Current Nevada Water Quality Standards

The current State of Nevada (2006 NAC 445A.144) molybdenum water quality standard (WQS) for protection of the aquatic life beneficial use is 19 µg/l, and was adopted based on recommendations contained in a report prepared by the California State Water Resources Control Board (CSWRCB, 1988) for the regulation of agricultural drainage to the San Joaquin River. The 19 µg/l standard was based on toxicity data from three studies (Birge et al., 1980; Birge, 1978; Kimball (MS)) and an ambient background concentration of molybdenum. The ambient background concentration of 0.68 µg Mo/L used was the average national surface water concentration reported by Hem (1970). The WQS was derived by taking the log mean of the three toxicity values and the background concentration. Thus, this criterion is strongly biased by the low ambient background concentration. The use of a background concentration in the derivation of the molybdenum WQS is not consistent with other EPA aquatic life criteria for toxics, nor is it toxicologically-based. Indeed, there are many aquatic life criteria that are much higher than typical background concentrations (e.g., zinc, ammonia, cyanide). The existing Nevada molybdenum standard was not developed using generally accepted criteria development methodology, nor was it developed using methods consistent with those recommended by the EPA. The molybdenum WQS should be based on current toxicological information, calculated using acceptable protocols (e.g., EPA-approved methodology), and the criteria should be relevant to species of concern in Nevada surface waters.

Nevada Division of Environmental Protection – Bureau of Water Quality Planning (NDEP-BWQP) has recognized that EPA has not developed national criteria for molybdenum to date. There are two reasons for this: (1) molybdenum is relatively rare in most parts of the country (hence the low national background concentration reported by Hem (1970), which was used by California) and therefore, it is not a high priority pollutant for most states; and (2) molybdenum is known to be much less toxic than many other metals or other inorganic substances and is not known to be either carcinogenic or otherwise constitute a major threat to ecological systems. Molybdenum is, however, relatively common and naturally occurs in Nevada waters (e.g., Table 1.1). Therefore, it is appropriate for Nevada to develop water quality criteria (WQC) for the protection of aquatic life for this element that is toxicologically based and developed in keeping with EPA recommendations for criteria development.

#### 1.4 Project Objectives

The objectives of this project were to develop appropriate molybdenum water quality criteria for aquatic life protection for the State of Nevada that are scientifically defensible, meet USEPA protocols for deriving criteria, and are based on available toxicological data and supporting information.

#### 2.0 Literature Review and Data Evaluation

A literature search was conducted to gather all available, relevant molybdenum toxicity data to freshwater aquatic life. This search included reviews of the following data sources:

- peer-reviewed scientific journals
- EPA's ECOTOX Database
- on-line subscription toxicology databases
- contacts with other researchers
- contacts with EPA regions and offices

The assembled studies were reviewed and toxicity data that was potentially useful in developing revised molybdenum criteria were assembled. Those studies that did not contain useful toxicity data or data for species not occurring in North America were not considered further. All other studies were reviewed in detail, as discussed below.

#### 3.0 Molybdenum Toxicity Data – Acute

Acute molybdenum toxicity data from 20 species were available (Table 3.1 and Table A-1). Those species for which data were available included 11 fish (four of which are salmonids), two planktonic crustaceans, an insect, a flatworm, a tubificid worm, a protistan, and three benthic invertebrates. The variety of organisms for which data are available appears to satisfy the "8 family rule", which outlines the minimum data requirements for development of a water quality criterion using EPA guidelines (Stephan et al. 1985). Further, these species include representatives from both warmwater (e.g., bluegill) and coldwater systems (e.g., rainbow trout) as well as several different invertebrate species that occupy diverse habitats. Therefore, available toxicity data are well suited for use in development of a state-wide water quality molybdenum standard for Nevada.

Species	Common Name
Catostomus commersoni	white sucker
Catostomus latipinnis	flannelmouth sucker
Ceriodaphnia dubia	cladoceran
Chironomus tentans	midge
Crangonyx pseudogracilis	isopod
Daphnia magna	cladoceran
Euglena gracilis	protistan
Esox lucius	northern pike
Gammarus fasciatus	scud
Girardia dorotocephala	flatworm
Hyalella azteca	amphipod
Ictalurus punctatus	channel catfish
Lepomis macrochirus	bluegill
Morone saxatilis	striped bass
Oncorhynchus kisutch	coho salmon
Oncorhynchus mykiss	rainbow trout
Oncorhynchus nerka	kokanee salmon
Oncorhynchus tshawytscha	chinook salmon
Pimephales promelas	fathead minnow
Tubifex tubifex	tubificid worm

**Table 3.1.** List of species for which acute molybdenum data are available.

### 3.1 Data Evaluation

In order to determine the suitability of the available toxicity data for use in developing a water quality criteria in general, and an appropriate standard for Nevada in particular, the data available for each species were evaluated. This evaluation focused on 1) the quality of the experimental methods used, 2) the documentation presented to support the reported toxicity values, and 3) the suitability of the selected experimental organisms to represent species which occur in Nevada. These evaluations largely followed recommendations of Stephan et al. (1985). Experimental methods were considered suitable if they were similar to those considered standard today (e.g., U.S. EPA and ASTM) regarding replication, environmental control (temperature and water quality), use of negative control treatments, etc. Documentation was considered suitable if enough information was presented with which to reproduce the study. Finally, organisms used in

testing were evaluated to determine whether the selected species occurs in Nevada or is a reasonable surrogate for organisms that occur in Nevada. For example, if a given species does not occur in Nevada, but the genus does occur, data from the species which does not occur should probably be included in the revised water quality criteria calculations. A table containing a summary of the results of these evaluations is contained in Appendix B (Table B-1). These evaluations are discussed below.

#### 3.1.1. Catostomus commersoni (white sucker)

Pyle (2000) evaluated the acute toxicity of molybdenum to *Catostomus commersoni* (white sucker). The author observed that exposure concentrations of up to 2,000 mg/L molybdenum resulted in no mortality to larval white sucker over a 96 hr exposure. As such, a 96 hr LC<sub>50</sub> of >2,000 mg/L was reported. The methods used by this author to generate this value appear reasonable and have been well described in Pyle (2000). Therefore, this acute value is appropriate for use in water quality criteria development. The species mean acute value (SMAV [calculated as a geometric mean in cases where more than one data point is available]) for *C. commersoni* is >2,000 mg/L.

Although white sucker does not occur in Nevada (Lee et al. 1980), several suckers in the genus *Catostomus* are native to Nevada (e.g., flannelmouth sucker, largescale sucker, and White River desert sucker). Therefore, it is appropriate that molybdenum data for white sucker be included in development of the molybdenum criteria.

#### 3.1.2. Catostomus latipinnis (flannelmouth sucker)

Hamilton and Buhl (1997) evaluated the acute toxicity of molybdenum to the flannelmouth sucker in simulated San Juan River water. The authors calculated a 96 hr LC<sub>50</sub> of 1,940 mg/L molybdenum for this species, which is in agreement with the LC<sub>50</sub> of >2,000 mg/L calculated for white sucker by Pyle (2000). The methods used were reasonable and well documented and this value should be appropriate for use in calculating a new water quality standard for molybdenum. Based upon this single acute toxicity value, the SMAV for flannelmouth sucker should be 1,940 mg/L.

Flannelmouth sucker do occur in Nevada (Lee et al. 1980). Therefore, it is appropriate to include toxicity data from this species in the development of water quality criteria for molybdenum for Nevada.

#### 3.1.3. Ceriodaphnia dubia (cladoceran)

Canton et al. (2007) determined the 48 hr molybdenum  $LC_{50}$  to *Ceriodaphnia dubia* to be 1,015 mg/L. The  $LC_{50}$  value presented in this manuscript is the geometric mean value of two replicate tests which generated  $LC_{50}$  values of 1,005.5 mg/L and 1,024.6 mg/L, indicating a high degree of precision in testing. These tests were conducted using the current EPA acute testing methods and are well described in the manuscript. Therefore, this value appears to be suitable for inclusion in criteria development. The SMAV for *C. dubia* should be set to 1,015.0 mg/L based upon the data in this study.

*C. dubia* are commonly used as a whole effluent toxicity (WET) test organism for NPDES compliance testing in Nevada. Further, members of the genus *Ceriodaphnia* are known to occur

in Nevada (e.g., Pyramid Lake). Because of their widespread use in WET testing as well as their occurrence in Nevada, it is appropriate to include data from *C. dubia* in development of molybdenum criteria.

#### 3.1.4. Chironomus tentans (midge)

Canton et al. (2007) calculated a 48 hr molybdenum  $LC_{50}$  to *Chironomus tentans* of 7,533.3 mg/L. This acute value is the geometric mean of two separate LC50 values calculated in this study (7,532.0 and 7,534.7 mg/L). The close agreement of these two test results indicate a high degree of precision associated with this value. These tests were conducted following ASTM methods and are well documented in the manuscript. The SMAV for this species is 7,533.3 mg/L based upon the data generated in this study.

This insect species is commonly used in sediment toxicity testing studies (e.g., EPA 2000). Further, this genus has been observed in several rivers in Nevada as part of Nevada Division of Environmental Protection (NDEP) monitoring efforts. It is not known whether or not *C. tentans* has been observed during these efforts because midges are not identified to species as part of this program. However, *C. tentans* should be included in the derivation of water quality criteria for molybdenum because the genus does occur in Nevada.

#### 3.1.5. Crangonyx pseudogracilis (isopod)

Martin and Holdich (1986) calculated a 96 hr LC<sub>50</sub> for molybdenum to the isopod, *Crangonyx pseudogracilis* of 2,650 mg/L. The methods used to calculate this value were well described in this publication and appear to be acceptable. Although the performance of the control organisms was not reported, the 95% confidence intervals around the LC<sub>50</sub> estimate are narrow (2,516 – 2,773 mg Mo/L), indicating a strong dose-response of the tested organisms and low mortality at low concentrations. The data, therefore, give little reason for poor organism performance and should be suitable for use. The SMAV for this species is 2,650 mg/L based upon this single acute value.

This genus has been observed in Nevada rivers during NDEP monitoring. However, individuals in this genus are not identified to species as part of this effort. Therefore, it is not known if *C. pseudogracilis* has been observed as part of these collections. Because this genus is known to occur in Nevada, it is appropriate to include data from this species in the molybdenum criteria development process.

### 3.1.6. Daphnia magna (cladoceran)

Acute molybdenum toxicity data were available from four different researchers for *Daphnia magna*. Kimball (1978) reported 48 hr LC<sub>50</sub> values of 206.8 and >400 mg Mo/L to *D. magna*. The test methods were adequately documented and appear to have been similar to those commonly accepted in contemporary toxicity studies. However, the performance of the control organisms was not documented and no confidence intervals were provided to give an indication of the precision of the LC<sub>50</sub> estimate. As such, it is impossible to determine whether these data are suitable for use in criteria development. Additionally, the higher of these two values was derived from an acute test in which the *D. magna* were fed, while the lower LC<sub>50</sub> value resulted from an unfed test. The standard protocol for acute testing with cladocerans (as per EPA 2002a)

is to not feed the organisms during testing. Based on this analysis, neither acute value generated by Kimball (1978) should be used in criteria development.

An additional study done by Bionomics (1973) reported a molybdenum  $TL_{50}$  for *D. magna* of 3,220 mg/L. The methods used in this study were well documented and similar to contemporary methodology. Additionally, control performance was adequately documented (100% survival).  $TL_{50}$  is the median tolerance limit which represents the concentration at which 50% of the test organisms survive. This measure of toxicity is not commonly used in contemporary toxicology and it is roughly equivalent to the  $LC_{50}$  which has taken its place as a measure of toxicity. Furthermore, Bionomics (1973) fed the *D. magna* in the test vessels. Therefore, this value should not be used in criteria development.

Diamantino et al. (2000) reported a 48 hr LC<sub>50</sub> of 2,847.5 mg/L for molybdenum to *D. magna*. This experiment followed appropriate toxicity testing methods and adequately documented both the methods and results. Although control performance was not documented, the 95% confidence intervals reported for this LC<sub>50</sub> estimate (2,838.7 – 2,857.0 mg Mo/L) indicate a strong dose-response and low mortality at lower test concentrations. This indicates that the test organisms likely performed adequately at lower concentrations and in the control concentration. Therefore, it is appropriate to include this value in the calculation of molybdenum criteria.

Canton et al. (2007) calculated a geometric mean 48 hr  $LC_{50}$  of 1,727.8 mg/L from two replicate tests which generated  $LC_{50}$  values of 1,776.6 and 1,680.4 mg/L, respectively. These test methods followed EPA protocols (EPA 2002a) and were adequately described in the manuscript. Control performance was documented and acceptable. Thus, these data are suitable for inclusion in the derivation of molybdenum criteria.

Based on these two acute values (2,847.5 and 1,727.8 mg/L), the SMAV (calculated as a geometric mean molybdenum acute value) for *D. magna* is 2,218.0871 mg/L.

*Daphnia magna* are commonly used in both WET and sediment toxicity testing of samples collected throughout the U.S., including Nevada (e.g., EPA 2000 and 2002a). Further, these organisms are widespread and common throughout the western U.S., including Nevada (EPA 2002a). Therefore, it is appropriate to include this species in development of the water quality standard for molybdenum.

### 3.1.7 Euglena gracilis (protistan)

Colmano (1973) exposed *Euglena gracilis* to a series of solutions containing elevated molybdenum concentrations. The growth media used to culture *E. gracilis* contains 5.44 mg Mo/L and was used as a control. Molybdenum concentrations of 96 mg/L were determined to result in abnormal cellular growth by the second day (24 hr) of exposure. After the same exposure period, cultures exposed to 960 mg Mo/L were observed to completely stop growing, which is equivalent to colony death for protists. The methods used in this study appear appropriate, adequately described to allow replication, and include documentation of acceptable control performance. Because abnormal growth is not an appropriate acute toxicity endpoint, the geometric mean of the control concentration and the concentration observed to completely stop growth (960 mg/L) was calculated to be 72.3 mg/L and used here as the acute value. Because

this is the only acute molybdenum toxicity value for *E. gracilis*, SMAV for this species should be 72.3 mg/L.

Members on the genus *Euglena* have a widespread distribution. It is likely that members of this genus and this species occur in Nevada. As such, toxicity data from this species should be included in criteria development.

#### 3.1.8. Esox lucius (northern pike)

Northern pike larvae exposed to 127.7 mg/L molybdenum had similar rates of survival to control fish not exposed to molybdenum (Pyle 2000). As such, the 96 hr  $LC_{50}$  for this species was reported to be >127.7 mg/L. The methods used in this test were reasonable and adequately documented. However, the control mortality reported for this study was 15-30%, which is excessive given that the typical mortality limit used in acute testing is 10%. As such, this value should not be included in derivation of molybdenum criteria for Nevada.

Although northern pike are not native to Nevada (Lee et al. 1980), they have been introduced and currently occur in the state. As such, if toxicity data of sufficient quality were available, it would be included in the derivation of water quality criteria for molybdenum for Nevada.

#### 3.1.9. Gammarus fasciatus (scud)

Bionomics (1973) generated a 48 hr TL<sub>50</sub> for *G. fasciatus* of 3,940 mg/L. The test methods used followed Standard Methods and appear to have been appropriate for use in generating molybdenum toxicity data. The control performance was documented and acceptable. Further, the documentation contained in the report is sufficient. Therefore, it appears that this acute value for *G. fasciatus* is appropriate for use in criteria development. Because this is the only value available for this species, the molybdenum SMAV should be 3, 940 mg/L.

*Gammarus fasciatus* has been collected in Nevada as part of NDEP's ambient bioassessment monitoring efforts. Because this species occurs in Nevada, it is appropriate to include data from this species in generating molybdenum criteria.

#### 3.1.10. Girardia dorocephala (flatworm)

Canton et al. (2007) conducted 96 hr acute testing with the flatworm, *Girardia dorocephala* using ASTM methods. This study generated two replicate 96 hr  $LC_{50}$  values of 1,334.2 and 1,125.8 mg Mo/L to *G. dorotocephala*. As with other tests described in this manuscript, the methods used appear suitable and the documentation of the experiment, adequate. The performance of the control organisms was documented and acceptable. Based upon this study, the SMAV for *G. dorocephala* for molybdenum should be 1,225.6 mg/L.

The species name for *G. dorotocephala* was previously *Dugesia dorotocephala* and is still referred to the older name in many references. *D. dorotocephala* has been observed in Nevada and should be included in the development of molybdenum water quality criteria.

### 3.1.11 Hyalella azteca (amphipod)

As part of a large screening-level toxicity study of 63 different metals and metalloids, Borgmann et al. (2005) conducted toxicity tests with the amphipod *Hyalella azteca*. The authors exposed

*H. azteca* to both sodium molybdate and ionic molybdenum from an atomic absorption standard as an acidified solution requiring pH buffering in low hardness (18 mg/L as CaCO<sub>3</sub>) and moderate hardness (124 mg/L as CaCO<sub>3</sub>) solutions. These tests resulted in LC<sub>50</sub> values of >1.0 mg/L in the soft water solution and for the sodium molybdate solution in moderately hard water and >3.15 mg/L for the atomic absorption standard solution in moderately hard water.

There were two major concerns raised in reviewing this study: 1) the study design and 2) the tested concentrations. First, the tests were designed to test acute toxicity, but were conducted for seven-days (168 hr) rather than the more typical 48 - 96 hr period (e.g., EPA 2002a and ASTM 2002). Additionally, during this seven-day period the test solutions were not renewed and the organisms were fed, both of which are deviations from more standard acute testing methods. Finally, the test method required only 80% survival of controls, which is less than the 90% survival required in a typical acute toxicity test. The final concern regarding this study are the extremely low molybdenum concentrations (1.0 and 3.15 mg/L) used in testing. These tested values were selected in an effort to categorize the tested metals per requirements of the Canadian Environmental Protection Act that uses a trigger of 1 mg/L in determining whether further assessment of a given substance is required. However, for molybdenum these tested concentrations are likely not toxicologically relevant. Based on this literature review, acute toxicity to invertebrates is one or more orders of magnitude higher than the concentrations tested in this study (Table 3.2). Therefore, including these estimated acute toxicity values as definitive values would unreasonably bias the resulting molybdenum water quality criteria. Thus, it is recommended that these data not be used in criteria derivation.

The amphipod *H. azteca* is known to occur in Nevada. Therefore, if usable toxicity data were available for this species, it should be included in the development of water quality criteria for molybdenum.

### 3.1.12 Ictalurus punctatus (channel catfish)

Bionomics (1973) generated a 96 hr TL<sub>50</sub> of >10,000 mg Mo/L to channel catfish. As discussed above for *G. fasciatus*, the TL<sub>50</sub> is roughly equivalent to the LC<sub>50</sub> and is an appropriate estimation of acute toxicity for use in criteria development. The methods used in testing the effects of molybdenum on channel catfish were appropriate (followed Standard Methods) and the documentation was sufficient. Additionally, the performance of control organisms was documented and acceptable. Therefore, the results of this study should be included in calculating revised criteria for molybdenum. Based on this acute molybdenum value for channel catfish, the SMAV for species is >10,000 mg/L.

Channel catfish are not native to Nevada, but have been introduced and established populations in the state (Lee et al. 1980). Therefore, this species should be included in the development of water quality criteria for molybdenum.

#### 3.1.13 Lepomis macrochirus (bluegill)

Bionomics (1973) exposed bluegill to sodium molybdate for 96 hr. This exposure resulted in a 96 hr  $TL_{50}$  value of 6,790 mg/L for bluegill. This exposure used the same methods as those for channel catfish, which appeared to have been appropriate and were well documented. The performance of the control organisms was documented and acceptable. Also, as discussed

above, the  $TL_{50}$  value is an appropriate measure of acute toxicity that is roughly equivalent to the  $LC_{50}$ . Therefore, this acute value is appropriate for use in criteria development. The resulting molybdenum SMAV for bluegill should be 6,790 mg/L.

Bluegill are not native to Nevada, but have been introduced and have established populations within the state (Lee et al. 1980). Therefore, this species should be included in the development of the water quality criteria for molybdenum.

#### 3.1.14. Morone saxatilis (striped bass)

Dwyer et al. (1992) evaluated the toxicity of molybdenum to striped bass and calculated a 96 hr  $LC_{50}$  of >79.8 mg/L. The methods used in testing were reasonable and well documented. Additionally, the performance of control organisms was documented and acceptable. However, this test was performed in synthetic laboratory water designed to mimic water quality conditions in the Stillwater Wildlife Management Area (SWMA) near Fallon, NV. As such, the salinity of this water was considerably higher than typical freshwater (~22 g/L). Because the test solutions were designed to simulate conditions observed in some ambient waters of Nevada (albeit in a small number of such waters), it is tempting to include this acute value in development of molybdenum criteria for the State. However, the use of saline dilution water in the test exposure makes this value unsuitable for use in freshwater criteria development.

Although striped bass are not native to Nevada, they have been stocked and are known to occur within the State (Lee et al. 1980). Therefore, it would be appropriate to include toxicity data from this species in the development of molybdenum criteria.

#### 3.1.15. Oncorhynchus kisutch (coho salmon)

Hamilton and Buhl (1990) exposed eyed eggs, alevins, and larvae of coho salmon to up to 1,000 mg/L molybdenum for 96 hr and observed no adverse effects. In fact, no mortality to any exposure concentration was observed. Therefore, these authors report a 96 hr  $LC_{50}$  of >1,000 mg/L for molybdenum to coho salmon. The experimental methods used were appropriate and properly documented. Although the authors did not report the performance of the control organisms, the fact that no mortality was observed in any treatment concentration indicates that neither the health of the organisms or test conditions negatively influenced the results of this experiment. Therefore, data from this study are suitable for use in criteria development. In that there are no other acute toxicity values available for coho salmon, the SMAV for this species should be set at >1,000 mg/L for molybdenum.

Coho salmon are not native to Nevada, but have been introduced into some waters of the State. Therefore, this species should be included in the development of water quality criteria for molybdenum.

#### 3.1.16. Oncorhynchus mykiss (rainbow trout)

Acute molybdenum data for rainbow trout were available from three separate sources. Similar to catfish and bluegill, Bionomics (1973) calculated  $TL_{50}$  values for rainbow trout using sodium molybdate in solutions with three different hardness values. For solutions with hardness values of 148, 154, and 290 mg/L as CaCO<sub>3</sub>,  $TL_{50}$  values of 7,340, 6,790 and 4,950 mg Mo/L were calculated. The different solutions used in testing may have had an influence on the final

toxicity, although there is no pattern relating hardness and toxicity as has been established for several other metals. These exposures were all performed following reasonable methods (based on Standard Methods) and were sufficiently documented. Likewise, the performance of organisms exposed to control treatments was documented and acceptable. Therefore, these acute values appear to be appropriate for use.

Goettl et al. (1976) calculated 96 hr molybdenum  $LC_{50}$  values of 800 mg/L and 1,320 mg/L for 25 mm and 55 mm rainbow trout, respectively. The methods used to generate these data are not well documented and insufficient to allow the experiments to be repeated by another researcher. These same data are presented by McConnell (1977) with detailed methods which are sufficient to allow for replication by another researcher. Based on the later presentation of these data by McConnell (1977), these acute data are appropriate for use in criteria development.

Pyle (2000) generated acute molybdenum toxicity data for larval and juvenile rainbow trout of >1,000 mg/L and >1,190 mg/L, respectively. Pyle (2000) used sodium molybdate and observed no mortalities in the highest molybdenum concentrations tested. As with other toxicity data presented by Pyle (2000), the methods used are reasonable and well documented. Although no control performance data were reported for these exposures, the fact that the author reported no mortality to any tested concentration indicates that organism health and test conditions were suitable. Therefore, each of the acute molybdenum values presented by Pyle (2000) should be considered appropriate for use.

Based upon the seven acute toxicity values determined to be appropriate for use, the calculated molybdenum SMAV for rainbow trout is 2,269.4034 mg/L.

Rainbow trout are native to Nevada and currently occur in the State (Lee et al. 1980). Thus, inclusion of this species in development of water quality criteria for molybdenum is appropriate.

### 3.1.17. Oncorhynchus nerka (kokanee salmon)

Reid (2002) exposed juvenile kokanee salmon (*Oncorhynchus nerka*) to molybdenum concentrations of up to 2,000 mg/L for 96 hr and observed no mortality. This study concluded that an appropriate 96 hr LC<sub>50</sub> for molybdenum for this species was >2,000 mg/L. The procedures used in testing were appropriate and well documented. The author did not report the performance of controls in the paper, but personal correspondence with Dr. Reid (University of British Columbia Okanagan) confirmed that control organisms experienced equal to or greater than 90% survival. As such, it is appropriate to use this acute value in criteria calculation. Because there are no other data from kokanee salmon, the SMAV for this species should be set at >2,000 mg/L.

Kokanee salmon are the land-locked form of sockeye salmon and have been extensively introduced in North America, including some waters of Nevada (Lee et al. 1980). While this species is not native to the State of Nevada, data from this species are appropriate for inclusion in molybdenum water quality criteria calculation as they are resident to at least some waters of the state.

### 3.1.18. Oncorhynchus tshawytscha (Chinook salmon)

Hamilton and Buhl (1990) exposed eyed eggs, alevins, and larvae of Chinook salmon to up to 1,000 mg/L molybdenum for 96 hr and observed no mortality or other adverse effects. Therefore, a 96 hr  $LC_{50}$  of >1,000 mg/L for molybdenum to Chinook salmon was calculated. The experimental methods used were appropriate and properly documented. As with coho salmon, these authors did not report the control performance. However, because there was no mortality in any exposure concentration, there is no concern that organism health or testing conditions negatively affected test outcome. Therefore, this acute molybdenum toxicity value should be used. Because this is the only acute molybdenum toxicity value available for Chinook salmon, the SMAV for this species should be set at >1,000 mg/L.

Chinook salmon are native to Nevada, but have not been observed in the State since collections made during the early 20<sup>th</sup> century in the Lahontan Basin. Because they are a native fish and taxonomically similar to other salmonids native to Nevada which still exist in the State (e.g., *O. clarkii*, cutthroat trout), it is appropriate to include data from this species in the development of water quality criteria for molybdenum.

#### 3.1.19. Pimephales promelas (fathead minnow)

Canton et al. (2007) generated a 96 hr molybdenum  $LC_{50}$  value 644.2 mg/L. The methods used in this exposure were appropriate and well documented. Further, the performance of the control organisms was documented and acceptable. Therefore, this acute value is appropriate for use in criteria development.

Pyle (2000) determined that exposure to 100 mg Mo/L resulted in no effects and presented a 96 hr  $LC_{50}$  of >100 mg/L. The methods and documentation presented are appropriate. Additionally, the performance of the control organisms in this experiment was documented and acceptable. Therefore, this acute molybdenum value appears to be suitable for use in molybdenum criteria development.

Kimball (1978) reported a 96 hr molybdenum  $LC_{50}$  value of 628 mg/L. However, no control performance data were reported. Therefore, it is not possible to assess the quality of these test data (no confidence intervals were reported with which to evaluate precision or dose-response relationship). Therefore, this value should not be used in criteria development.

Bionomics (1973) calculated a 96 hr TL<sub>50</sub> of 7,630 mg/L, which is more 10 times higher than the value of 644.2 mg/L presented by Canton et al. (2007). The fact that this value is so much greater than others calculated for fathead minnow is of concern. However, the methods provided appear sound, were well documented, and control performance was adequate. Given that EPA guidance recommends against using data with greater than a 10-fold difference in toxicity, it is best to exclude this value from use.

Tarzwell and Henderson (1960) present 96 hr  $LC_{50}$  values of 70 mg/L and 370 mg/L at hardness values of 20 and 400 mg/L as CaCO<sub>3</sub>, respectively. The results of this study suggest that hardness has some influence on the toxicity of molybdenum. Although such a relationship is well established for other metals (e.g., copper), no other acute data reviewed in this effort support this relationship. The report by Tarzwell and Henderson (1960) is brief (one page),

gives little information as to test methods used, and does not report whether controls were used. Therefore, data from this study should not be used in criteria development.

Based on the data from the two studies determined to be acceptable, the molybdenum SMAV for fathead minnow is 253.8110 mg/L.

Fathead minnows are not native to Nevada. However, they have become established in the state (Lee et al. 1980). Therefore, inclusion of data from this species is appropriate for calculation of water quality criteria for molybdenum.

#### 3.1.20 Tubifex tubifex (tubificid worm)

Khangarot (1991) conducted 96 hr acute exposures of the oligochaete, *Tubifex tubifex* to molybdenum as sodium molybdate. The author calculated a 96 hr  $EC_{50}$  using immobilization as the endpoint of 28.91 mg Mo/L. The experimental methods used in generating this value were well documented and appropriate. Although, no control survival data was reported for this study, the study did note that organisms in control tests "remained active during the test period." Based on this review, the data presented by Khangarot (1991) is appropriate for use in criteria development and the SMAV for this species should be set at 28.9100 mg/L.

Tubificid worms are broadly distributed and have been reported to occur in Nevada. Therefore, inclusion of data from this species is appropriate for calculation of water quality criteria for molybdenum.

#### 3.2 Species Mean Acute Values

Following a review of the available acute toxicity data, data from 17 of the 20 species identified in Section 3.1 have been determined to be suitable for use in establishing appropriate water quality criteria for molybdenum for Nevada. These 17 species are sufficient to satisfy the minimum data requirements set forth by Stephan et al. (1985) for generation of water quality criteria. These data are listed in Table 3.2 as SMAV and ranked according to species sensitivity from least to most sensitive to acute molybdenum toxicity. In keeping with Stephan et al. (1985) methodology, all SMAVs are reported using four decimal places to prevent rounding error and all values recorded as "greater than" a test value are recorded here as the actual value for purposes of criteria calculation (e.g., Chinook, coho, and kokanee salmon). The four most sensitive species from most to least sensitive are Chinook and coho salmon (SMAV = 1,000.0000 mg/L), fathead minnow (SMAV = 253.8110 mg/L), *E. gracilis* (SMAV = 72.3000 mg/L), and *T. tubifex* (SMAV = 28.9100 mg/L).

			SMAV
Rank	Species Common Name		(mg/L)
15	Ictalurus punctatus	channel catfish	10,000.0000
14	Chironomus tentans	midge	7,533.3000
13	Lepomis macrochirus	bluegill	6,790.0000
12	Gammarus fasciatus	scud	3,940.0000
11	Crangonyx pseudogracilis	isopod	2,650.0000
10	Oncorhynchus mykiss	rainbow trout	2,269.4034
9	Daphnia magna	cladoceran	2,218.0871
8	Oncorhynchus nerka	kokanee salmon	2,000.0000
8	Catostomus commersoni	white sucker	2,000.0000
7	Catostomus latipinnis	flannelmouth sucker	1,940.0000
6	Girardia dorotocephala	flatworm	1,225.6000
5	Ceriodaphnia dubia	cladoceran	1,015.0000
4	Oncorhynchus kisutch	coho salmon	1,000.0000
4	Oncorhynchus tshawytscha	chinook salmon	1,000.0000
3	Pimephales promelas	fathead minnow	253.8110
2	Euglena gracilis	protistan	72.3000
1	Tubifex tubifex	tubificid worm	28.9100

**Table 3.2.** List of species for which molybdenum acute toxicity data are available. Species are ranked in order of sensitivity by species mean acute value (SMAV).

#### 4.0 Molybdenum Toxicity Data – Chronic

Chronic molybdenum toxicity data are available from eight species, including five fish (one salmonid; rainbow trout), two planktonic crustaceans (cladocera), and a toad (Table 4.1 and Table A-2). It does not appear that data are available from a sufficient diversity of species to develop a chronic standard independent of the acute standard using EPA methods (Stephan et al. 1985). In order to develop such an independent standard, data from an insect and a benthic crustacean are required, which are not currently available for molybdenum. However, it does appear that sufficient data are available for use in developing a chronic standard using an acute-to-chronic ratio (ACR). This method requires the development of a relationship between acute and chronic data from three different species, which are available in the acute and chronic data sets.

Species	Common Name	
Carassius auratus	goldfish	
Catostomus commersoni	white sucker	
Ceriodaphnia dubia	cladoceran	
Daphnia magna	cladoceran	
Esox lucius	nothern pike	
Gastrophryne carolinensis	narrow-mouthed toad	
Oncorhynchus mykiss	rainbow trout	
Pimephales promelas	fathead minnow	

Table 4.1.	List of species for which chronic molybdenum
toxicity dat	ta are available.

#### 4.1 Data Evaluation

As with the acute molybdenum data reviewed in Section 3.1, the chronic molybdenum data review focused on the experimental methods used to generate the toxicity data and the documentation of the study to ensure that reasonable methods (based upon test methods published by EPA and ASTM, where available) were used to generate data and sufficient documentation is available to review and repeat the study, if necessary. Finally, the appropriateness of each species for use in generating a water quality standard for Nevada was reviewed. A table containing a summary of the results of these evaluations is contained in Appendix B (Table B-2).

#### 4.1.1. Carassius auratus (goldfish)

Birge (1978) exposed goldfish eggs to molybdenum from fertilization until four days post-hatch, which resulted in a total exposure of seven days. This exposure resulted in a seven-day survival  $LC_{50}$  of 60.0 mg/L. The methods used in this study appear reasonable and are reasonably well documented. However, no control performance data are available making it impossible to assess the quality of the test organisms. Additionally, the short duration of the test exposure is of concern in applying the resulting  $LC_{50}$  data in development of a chronic criterion. EPA guidance on criteria development (Stephan et al. 1985) states that chronic values for fish should be derived from tests initiated with eggs with exposure continued for no less than 24 days post-hatch for most fish (90 days post-hatch for salmonids). Because Birge (1978) only exposed the fish for

four days post-hatch, these test methods do not meet the recommendations of EPA. Therefore, it is not appropriate to include this value in deriving molybdenum criteria for Nevada.

Goldfish are not native to Nevada, but have been introduced and have reproducing wild populations in the State (Lee et al. 1980). Thus, appropriate chronic toxicity data for goldfish would be suitable for use in calculating molybdenum criteria for Nevada.

#### 4.1.2. Catostomus commersoni (white sucker)

In addition to generating acute toxicity data for white sucker, Pyle (2000) exposed fertilized white sucker eggs to molybdenum using ASTM methods. Tests were conducted for 22 days, which was just before larvae initiated exogenous feeding. Pyle (2000) observed no impacts to the hatchability of eggs after 12 days of exposure and no impacts to larval growth after 22 days of exposure to molybdenum concentrations as high as 1.7 mg/L. The methods were well documented and appropriate and control performance was acceptable. The only concern in using these data was that exposure period was short of the required 24 days post-hatch. However, in that the 22 day exposure period approaches the 24 days post-hatch requirement, it is suggested that these endpoints should be included in the final derivation. The endpoints calculated at 12 days should be discounted for the purposes of developing chronic criteria. Given that the 22 day larval growth NOEC is 1.7 mg/L and the LOEC and IC<sub>25</sub> for larval growth are both >1.7 mg/L, the species mean chronic value (SMCV, calculated as a geometric mean, similar to the SMAV) for white sucker should be >1.7 mg Mo/L.

As discussed in Section 3.1, white sucker do not occur in Nevada, but are reasonable surrogates for other fish in this genus that do occur. Therefore, inclusion of data from white sucker is appropriate in water quality criteria development.

### 4.1.3 Ceriodaphnia dubia (cladoceran)

Naddy et al. (1995) reported  $IC_{25}$  and  $IC_{50}$  values of 47.5 mg/L and 79.7 mg/L, respectively for *C. dubia* exposed to molybdenum for seven days. Similarly, Canton et al. (2007) reported an  $EC_{20}$  of 76.9 mg/L for *C. dubia* exposed to molybdenum for seven days. In addition, Canton et al. (2007) calculated an NOEC of 0 mg/L and an LOEC of 76.3 mg/L for this same exposure. As with other species, the endpoints reported by Canton et al. (2007) are the geometric mean of two replicate tests. Both studies used appropriate methods for calculating these results and adequately documented the experiments. Canton et al. (2007) reported adequate control performance and Naddy et al. (1995) cited a test method that requires a minimum acceptable control performance. Therefore, both studies are suitable for use in criteria development.

Because the  $EC_{20}$  is the preferred measure of chronic toxicity for use in calculating water quality criteria (e.g., EPA 2007), the  $EC_{20}$  calculated by Canton et al. (2007) of 76.9 mg/L molybdenum should be used in criteria development. Similarly, the  $IC_{25}$  value reported by Naddy et al. (1995) is more conservative than the  $IC_{50}$  value and more closely approximates the  $EC_{20}$  value. Thus, the  $IC_{25}$  value of 47.5 mg/L reported by Naddy et al. (1995) should be used in development of criteria. The geometric mean of these two selected values is 60.44 mg/L, which should be the SMCV for *C. dubia* for molybdenum.

Although the *C. dubia* test methods in the above studies used seven days of exposure rather than the 21 day exposure discussed by Stephan et al. (1985) for Daphnids, these test results are appropriate for use. The standard EPA *C. dubia* chronic test method (EPA 2002b) requires 6-8 days of exposure (test duration is dependent upon control reproduction). Further, chronic tests of this duration and shorter have been previously used by EPA to develop national water quality criteria (e.g., EPA 2007).

As discussed in Section 3.1, *C. dubia* are commonly used as a whole effluent toxicity (WET) test organism for NPDES compliance testing in Nevada. Further, members of the genus *Ceriodaphnia* are known to occur in Nevada (e.g., Pyramid Lake). Because of their widespread use in WET testing as well as their occurrence in Nevada, it is appropriate to include data from *C. dubia* in development of molybdenum criteria.

#### 4.1.4 Daphnia magna (cladoceran)

Three different studies have produced chronic molybdenum toxicity data for D. magna based on exposures of 28 days (Kimball 1978) and 21 days (both Diamantino et al. 2000 and Canton et al. 2007). All three studies used appropriate methods and documented those methods sufficiently. Additionally, the control performance was reported as adequate in each study. Kimball (1978) generated a 28 day LC<sub>50</sub> of 0.67 mg/L and an LOEC and NOEC based on reproduction of 1.15 mg/L and 0.67 mg/L, respectively. Another measure of the chronic value that is used in criteria development (e.g., EPA 2007) when there is not a point-estimate of the chronic effect value (e.g.,  $EC_{20}$  or  $IC_{25}$ ) is the geometric mean of the NOEC and LOEC. This chronic value molybdenum for the Kimball (1978) D. magna data is 0.87 mg/L. Diamantino et al. (2000) reported LOEC and NOEC values for reproduction of 75 mg/L and 50 mg/L, respectively. Additionally, these authors reported an EC<sub>50</sub> of 102.1 mg/L. Because using the EC<sub>50</sub> value would be less conservative than those values used in comparable tests, the geometric mean of the LOEC and NOEC (61.2 mg/L) should be used as the chronic value. Canton et al. (2007) calculated an EC<sub>20</sub> of 153.8 mg/L based upon two replicate tests which produced  $EC_{20}$  values of 146.7 mg/L and 161.2 mg/L. Additionally, Canton et al. (2007) determined geometric mean LOEC and NOEC values of 270.6 mg/L and 136.1 mg/L for these two D. magna tests.

The appropriate endpoints for use in criteria development from each of these studies are the geometric mean of the NOEC and LOEC from Kimball (1978) of 0.87 mg/L, the geometric mean of the NOEC and LOEC from Diamantino et al. (2000) of 61.2 mg/L, and the  $EC_{20}$  from Canton et al. (2007) or 153.8 mg/L. While the chronic values presented by Diamantino et al. (2000) and Canton et al. (2007) are fairly similar in that they differ by a factor of 2.5, the chronic value determined by Kimball (1978) is much lower.

There are two substantive differences in the methods used in these three experiments: 1) test duration and 2) tested material. The Kimball (1978) test lasted 28 days, while tests in the other two studies lasted 21 days. However, all tests were sufficiently long to elicit a toxic response and the more recent test was performed in keeping with widely accepted methodology (i.e., ASTM). Therefore, it is unlikely that test duration was responsible for the observed differences in reported effect. The second difference is that Kimball (1978) tested the toxicity of molybdenum as molybdenum oxide, whereas the later two studies used sodium molybdate. In waters with pH values in excess of 7.0, both molybdenum oxide and sodium molybdate would be

expected to result in a molybdenum solution consisting primarily of  $MoO_4^{2-}$ , the molybdate ion (Eisler 1989). It does not appear that these two different molybdenum compounds should result in substantially different toxic responses based on the simple chemistry.

While the striking difference in reported chronic molybdenum toxicity to *D. magna* between the older (Kimball 1978) and more recent (Diamantino et al. (2000) and Canton et al. (2007)) is odd, there is no obvious differences in the methods used that suggest are reason for the observed differences. Stephan et al. (1985) recommends that acute toxicity data that differs by a factor of 10 or more for the same species should not be used. Because, the Kimball (1978) value is so much lower than all other values for not only cladocerans, but all other chronic values with the exception of a single other value reported by Kimball (1978) for the narrow-mouthed toad (see below), it should be discarded and not used in standard development. As such, the SMCV should be calculated using the chronic values from Diamantino et al. (2000) and Canton et al. (2007), which results in a SMCV for *D. magna* of 97.02 mg/L.

As discussed in Section 3.1, *D. magna* are commonly used in both WET and sediment toxicity testing of samples collected throughout the U.S., including Nevada (e.g., EPA 2000 and 2002a). Further, these organisms are widespread and common throughout the western U.S., including Nevada (EPA 2002a). Therefore, it is appropriate to include this species in development of the water quality criteria for molybdenum.

### 4.1.5 Esox lucius (northern pike)

Pyle (2000) exposed northern pike larvae to sodium molybdate for 13 days to determine potential impacts on growth and survival. The methods (ASTM) used appear to have been appropriate for generation of short-term chronic toxicity data and were well described. No effects were observed at 1.7 mg Mo/L, the highest concentration tested. Therefore, the author reported an  $IC_{25}$  value of greater than 1.7 mg/L as well as an NOEC of 1.7 mg/L and an LOEC of >1.7 mg/L.

Although the methods were acceptable for generation of data and well described, the methods used do not comply with guidance outlined by Stephan et al. (1985) for generation of chronic toxicity data for fish. Stephan et al. (1985) indicates that early life-stage tests such as this one should be conducted for a minimum of 28 days, while this experiment included an exposure duration of only 13 days. Therefore, these data should not be included in criteria development.

As discussed in Section 3.1, northern pike are not native to Nevada (Lee et al. 1980), but have been introduced and currently occur in the state. As such, valid chronic toxicity data for northern pike are appropriate for use in derivation of water quality criteria for molybdenum for Nevada.

#### 4.1.6 Gastrophryne carolinensis (narrow-mouthed toad)

Birge (1978) exposed eggs of the narrow-mouthed toad to molybdenum until four days after egg hatching for a total exposure period of seven days. Birge (1978) calculated a 7-day  $LC_{50}$  for survival of 0.96 mg/L for the toad based upon this exposure. The methods used to generate this toxicity value appear reasonable, but are not sufficiently described to allow this study to be replicated. Further, no control performance data are provided to use in evaluating the quality of this toxicity value. The short duration of this exposure is also of concern, as seven days is much shorter than acceptable test durations for other vertebrates. Therefore, this chronic value should

not be used in criteria development. Given that this toxicity value is surprisingly low compared to all other toxicity values determined to be acceptable for use, there is some concern in not including it in the revised criteria. Further studies into the susceptibility of amphibians to molybdenum would be a useful endeavor for future research.

The narrow-mouthed toad does not occur in Nevada, nor do any other members of the genus Gastrophryne or the family Microhylidae. All frogs and toads that occur in Nevada are members of the order Anura, which also contains narrow-mouthed toads. Therefore, inclusion of data from the narrow-mouthed toad would be questionable based upon distribution. Any future research on molybdenum toxicity to amphibians would be best performed on a more widely distributed species or one that occurs in Nevada.

#### 4.1.7 Oncorhynchus mykiss (rainbow trout)

Three studies were available that produced chronic molybdenum toxicity data for rainbow trout. The first study was a long-term (18 month) study, the next was a 28 day study, and the third was a repeat of the 28 day study.

Goettl et al. (1976) reported that a partial life-cycle exposure (18 months) of rainbow trout to molybdenum concentrations of up to 18.5 mg/L resulted in no adverse effects. The exposure was initiated on eggs and was of sufficient duration for inclusion based upon guidance by Stephan et al. (1985). The methods used were documented in the Goettl et al. (1976) report and in an earlier report (Goettl and Davies 1975). However, the descriptions of these methods were insufficient to allow this study to be repeated. Further, no control data are provided with which to evaluate the health and performance of unexposed fish. Therefore, this value should not be used in criteria development.

McConnell (1977) also reported results of a partial life-cycle exposure of rainbow trout to molybdenum concentrations up to 17 mg/L for one year. In many ways, including the molybdenum dilution series, this study was very similar to that performed by Goettl et al. (1976). Given that acute data presented by McConnell (1977) were identical, but more thoroughly reported, to those presented by Goettl et al. (1976, see Section 3.1), it is likely that the study described by McConnell (1977) is identical to that presented by Goettl et al. (1976), although the later publication makes no mention of the earlier author or publication. The exposures were initiated with eyed eggs and showed no reductions in growth or mortality relative to control performance. The methods used appear appropriate and were fairly well documented. However, this study did not produce a definitive chronic toxicity endpoint. The fact that no definitive toxic endpoint (see discussion of Davies et al., 2005 below), makes the results of this study unsuitable for use in criteria development.

Birge (1978) reported a seven day survival  $LC_{50}$  of 0.96 mg/L on rainbow trout. The methods used in this study appear appropriate, but were not sufficiently documented to allow the study to be repeated if necessary. Further, no control data were provided for this experiment. Finally, as the study duration was shorter than suggested by Stephan et al. (1985), this value should not be used in criteria development.

Birge (1978) also provided a 28 day  $LC_{50}$  of 0.73 mg Mo/L for rainbow trout exposed as eggs until a point four days post-hatch. This study used reasonable methods that were sufficiently documented to allow this study to be repeated. However, no control performance data were included. Therefore, it is impossible to evaluate the quality of this chronic value.

Birge et al. (1980) presented another 28 day  $LC_{50}$  for molybdenum of 0.79 mg/L using the same methods as those used in his 1978 study presented above. This study did include control performance data indicating acceptable survival. Similar to Birge's 1978 study, there is little information presented in this study with which to evaluate the quality of these data. However, there is not sufficient information to reject these data based on the study alone.

Davies et al. (2005) repeated the 28 day exposures presented in the Birge (1978) and Birge et al. (1980) studies in an attempt to duplicate the reported effects as well as to generate toxicity data using standard Environment Canada methods, which are equivalent to EPA methods. Davies et al. (2005) observed insufficient mortality to generate an  $LC_{50}$  at molybdenum concentrations of up to 400 mg/L using the methods of Birge (1978) and Birge et al (1980). However, a significant decrease in survival was observed at 400 mg/L, relative to lower tested concentrations. Thus, an NOEC of 200 mg/L and an LOEC of 400 mg/L were reported by Davies et al. (2005) for this test using the Birge (1978) and Birge et al. (1980) methods. In the test exposure using Environment Canada methods, Davies et al. (2005) reported much higher survival rates with an LOEC of 1,000 mg/L and an NOEC of 750 mg/L. The authors attributed the increased survival between the two different test methods to the way the trout eggs were handled using Birge (1978) and Birge et al. (1980) methods, which exposed the eggs to additional stress. Based upon the Davies et al. (2005) results, it appears that a molybdenum chronic value of 866.0 mg/L (geometric mean of the NOEC and LOEC from the second Davies et al. test) should be used to the exclusion of either the Birge (1978), Birge et al. (1980), or the repeated Birge (1978) and Birge et al. (1980) data generated by Davies et al. (2005).

The fact that the partial life-cycle exposure reported by Goettl et al. (1976) and McConnell (1977, likely the same study) far exceeded the exposure duration of the Davies et al. (2005) study makes these values attractive for use in calculating the SMCV for rainbow trout. The partial life-cycle study is also in keeping with EPA recommendations (Stephan et al. 1985) on selection of data for use in criteria development. However, the poor documentation and lack of control data (Goettl et al. 1976) and lack of a definitive endpoint (McConnell 1977) exclude these values from use in criteria development. Therefore, the chronic data from Davies et al. (2005) is used and the molybdenum SMCV for rainbow trout is 866.0254 mg/L.

As discussed in Section 3.1, rainbow trout occur in Nevada. Thus, inclusion of data from this species in criteria development efforts is appropriate.

### 4.1.8 Pimpephales promelas (fathead minnow)

Pyle (2000) reported that fathead minnow egg hatchability and time-to-hatch were not affected by exposure of up to 100 mg Mo/L for up to 96 hours. Additionally, fathead minnow growth was not affected by seven day exposure to up to 100 mg Mo/L. The methods used by Pyle in generating these data were appropriate and well documented. However, the duration of these

tests was too short to satisfy recommendations set out by EPA (Stephan et al. 1985) for chronic test duration. Therefore, these data should not be used in criteria development. Canton et al. (2007) exposed fathead minnow to molybdenum for 28 days post-hatch in replicate tests. These two tests resulted in growth NOEC and LOEC values of 143.8 mg/L and 258.4 mg/L, respectively for both tests. Additionally, Canton et al. (2007) reported growth EC<sub>20</sub> values of 162.0 mg/L and 165.1 mg/L, which equal a geometric mean chronic value of 163.5 mg/L. The methods used by Canton et al. (2007) are appropriate and well documented and in keeping with recommended test method and duration issued by EPA. Additionally, Canton et al. (2007) reported acceptable control performance. Therefore, the molybdenum SMCV for fathead minnow should be 163.5427 mg/L.

As discussed in Section 3.1, fathead minnow is not native to Nevada, but has become established in the State (Lee et al. 1980). Therefore, inclusion of data from this species in criteria development is appropriate.

#### 4.2 Species Mean Chronic Values

Based on this review of the available chronic molybdenum toxicity data, data from five of the eight of the species identified in Section 4.1 have been determined to be suitable for use in establishing water quality criteria for molybdenum for Nevada. These data are listed in Table 4.2 as SMCV and ranked according to species sensitivity to molybdenum from least to most sensitive. As with the SMAV values in section 3.2, these SMCV values are reported using four decimal places as required by EPA guidance (Stephan et al. 1985). Additionally, all "greater than" values are recorded as the highest tested concentration for purposes of SMCV calculation. Thus, although the chronic test for white sucker found no effects at the highest tested concentration of 1.7 mg Mo/L, this species becomes the most sensitive SMCV by virtue of the low test concentration. The four most sensitive species from least to most sensitive are fathead minnow (SMCV = 163.5427 mg/L), *D. magna* (SMCV = 97.0183 mg/L), *C. dubia* (SMCV = 60.4380 mg/L), and white sucker (SMCV = 1.7000 mg/L).

Rank	Species	Common Name	SMCV (mg/L)
5	Oncorhynchus mykiss	rainbow trout	866.0254
4	Pimephales promelas	fathead minnow	163.5427
3	Daphnia magna	cladoceran	97.0183
2	Ceriodaphnia dubia	cladoceran	60.4380
1	Catostomus commersoni	white sucker	1.7000

**Table 4.2.** List of species for which molybdenum chronic toxicity data are available. Species are ranked in order of sensitivity by species mean chronic value (SMCV).

As discussed in Section 4.0, there are not sufficient chronic molybdenum data to calculate a chronic criterion independent of the acute criterion. However, there are matched acute and chronic data for all five species in the final chronic dataset. Therefore, use of the acute-to-chronic ratio for chronic criterion calculation is possible.

#### 5.0 Criteria Development Rationale and Methodology

As discussed in Section 1.0, the current Nevada molybdenum standard for protection of aquatic life was not developed through the use of standard or EPA-approved methods for development of water quality criteria for the protection of aquatic life. Further, the aquatic toxicity data for molybdenum to aquatic life relevant to the State of Nevada presented in Sections 3.0 and 4.0 demonstrate that the toxicity data used in developing the existing Nevada molybdenum standard was developed using extremely limited data relative to the current state of knowledge on the toxicity of molybdenum to aquatic life. Based upon these two findings, it is clear that development of molybdenum criteria for protection of aquatic life for the State of Nevada using EPA-approved methods and current molybdenum toxicity data is appropriate.

#### 5.1 Method Discussion

The standard protocol for use in deriving water quality criteria for toxic chemicals is EPA's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and their Uses* (Stephan et al. 1985). This is the guidance used to develop most of the current EPA criteria for metals. As discussed above, this guidance sets the minimum data requirements (MDRs) for developing criteria (Table 5.1) as well as the procedures to follow for calculating criteria based upon those data. Additionally, this guidance discusses the types of toxicity tests appropriate for use in generating data to be used in criteria development (again, as discussed above).

**Table 5.1.** Minimum Data Requirements (MDR) set forth in EPA criteria development guidance (Stephan et al. 1985). The species for which toxicity data are included in the database for use in criteria development must include at least one representative from each category outlined here.

Requirement #	<b>Requires a representative from:</b>		
1	Member of the family Salmonidae		
2	Member of another family in class Osteichthyes		
3	A third family in phylum Chrordata		
4	A planktonic crustacean		
5	A benthic crustacean		
6	An insect		
7	A family in a phylum other than Arthropoda or Chordata		
8	A family in any order of insect or any phylum not already represented		

In addition to the standard protocols for use in deriving water quality criteria, there are several other methods that have been used. The data available for or the toxic action of some compounds do not suit the methods outlined by Stephan et al. (1985) for criteria development. For instance, insufficient data may be available to meet the MDRs for a given compound, but criteria are clearly needed to protect aquatic life uses from toxic effects. The Great Lake Initiative (GLI) promulgated by EPA allowed for the development of "Secondary Criteria", when insufficient species data are available to calculate a national criterion. The GLI uses safety factors, depending on the number of acute toxicity data requirements satisfied to ensure resulting criteria are adequately protective. Similarly, the GLI uses a default ACR of 18 in cases where

the three ACR values required using standard EPA criteria development methodology (Stephan et al. 1985) are not available.

The toxicity of some compounds (e.g., bioaccumulative toxins such as selenium or mercury) may not be apparent in standard laboratory testing scenarios advocated by Stephan et al. (1985). Special criteria development methods must be used to develop adequately protective criteria for such compounds. Finally, if a large amount of data have been generated with which to develop a predictive understanding of the impacts of various other water quality characteristics (e.g., pH, dissolved organic carbon) on the toxicity of a compound (e.g., copper) other methods such as the Biotic Ligand Model may be used to develop criteria. Such methods allow more detailed criteria to be calculated that can more accurately taken into account site-specific water quality conditions that may alter the toxicity of a given compound.

#### 5.2 Method Selection

Based upon the data gathered and reviewed in Sections 3 and 4, there are sufficient acute molybdenum toxicity data to satisfy the minimum data requirements outlined by Stephan et al. (1985). Each of the MDR numbers listed in Table 5.1 above is listed again in Table 5.2 with the species which satisfies that data requirement (some requirements are satisfied by more than one species). Based on this analysis, sufficient acute toxicity data are available and appropriate for use in deriving a revised acute molybdenum criterion.

**Table 5.2.** Listing of the eight minimum data requirements outlined by Stephan et al. (1985) for criteria development and the species contained in the acute molybdenum toxicity database which satisfy these requirements.

Requirement #	<b>Requirement Satisfied by:</b>		
1	Chinook salmon, coho salmon, kokanee salmon, and rainbow trout		
2	Bluegill		
3	Flannelmouth sucker, white sucker, channel catfish, fathead minnow		
4	Ceriodaphnia dubia (cladoceran), Daphnia magna (cladoceran)		
5	Gammarus fasciatus (scud), Crangonyx pseudogracilis (isopod)		
6	Chironomus tentans (midge)		
7	<i>Girardia dorotocephala</i> (flatworm)		
8	Euglena gracilis (protistan), Tubifex tubifex(tubificid worm)		

For derivation of a chronic criterion using EPA methods (Stephan et al. 1985), either an independent criterion based upon a chronic toxicity database which meets the eight family rule can be developed or a ratio between acute and chronic toxicity for three or more species, termed the acute-to-chronic ratio (ACR) can be developed. The ACR is calculated by determining the ratio of acute to chronic toxicity for species for which both approved acute and chronic data are available. Because acute toxicity data is available for each of the five species for which approved chronic toxicity data is available, sufficient data exist to calculate a chronic criterion through use of the ACR.

The toxicity of molybdenum in aquatic systems does not appear to be related to bioaccumulation (Section 9.0, below) and there is no apparent relationship between the hardness of the solution and the toxicity of molybdenum as has been observed for many other metals. Additionally,

sufficient molybdenum toxicity data exist to satisfy the MDRs of EPA standard criteria development methodology (Stephan et al 1985). Because the EPA approach outlined by Stephan et al. (1985) is the standard approach to criteria development and sufficient data exist with which to use this approach, it is recommended that molybdenum criteria be developed using these methods.

### 6.0 Acute-to-Chronic Ratio (ACR)

The ACR is calculated by determining the ratio of acute to chronic toxicity for species for which both approved acute and chronic data are available. Because acute toxicity data are available for each of the five species for which approved chronic toxicity data are available, sufficient data exist to calculate a chronic criterion through use of the ACR. Stephan et al. (1985) state that ACR values for at least three species in three different families should be available for final ACR calculation. Further, this guidance indicates that ACR values should be calculated from acute and chronic toxicity data that were generated as part of the same study (e.g., same dilution water and same laboratory).

#### 6.1 ACR Calculation

As discussed above, acute and chronic molybdenum toxicity data were available for each of the five different species for which chronic toxicity data are available (Table 4.2). Acute and chronic molybdenum toxicity data were generated in the same laboratory and using the same dilution water for *Ceriodaphnia dubia*, fathead minnow, and *Daphnia magna*, (Table 6.1). However, both *C. dubia* and *D. magna* are in the same family (Daphniidae), which requires that ACR data generated for a species in a third family be used in final ACR calculation. Acute and chronic data suitable for use in ACR calculation were available for rainbow trout, but were generated by different laboratories. The ACR value for rainbow trout was calculated based on the SMAV and SMCV and included in final ACR calculation to satisfy the requirement that ACR data from species representing three different families be used in criteria development.

Species	Acute Value (mg/L)	Chronic Value (mg/L)	ACR	Species Mean ACR	Reference
Ceriodaphnia dubia	1,015.0	76.9	13.2	13.2	Canton et al. 2007
Pimephales promelas	644.2	163.5	3.9	3.9	Canton et al. 2007
Daphnia magna	1,727.8	153.8	11.2	22.9	Canton et al. 2007
Daphnia magna	2,847.5	61.2	46.5		Diamantino et al. 2000
Oncorhynchus mykiss	2,269.4	866.0	2.6	2.6	Various
Final ACR				7.5	

**Table 6.1.** Summary of acute and chronic molybdenum toxicity values used in calculation of acute-to-chronic ratios (ACR) and subsequent ACR data.

ACR values were determined for each species and the geometric mean ACR was calculated for *D. magna* as it was the only species for which two individual ACR values were able to be calculated from two independent studies. Additionally, an ACR of 1,176.5 was calculated for white sucker (*Catostomus commersoni*), but excluded from use in calculating the final mean ACR. Both acute (>2,000 mg/L) and chronic (>1.7 mg/L) molybdenum toxicity values for white sucker were "greater than" values indicating that the concentration listed was the highest concentration tested and determined to elicit no toxic response. Therefore, the calculated ACR for this species is an artifact of selected test concentrations and not representative of an actual relationship between acute and chronic sensitivity of white sucker to molybdenum toxicity.

The final ACR values ranged from 2.6 to 22.9 for each species, with a final geometric mean ACR of 7.5 for molybdenum. As per EPA criteria development guidance, this final ACR value was used in calculation of the chronic water quality criterion.

#### 7.0 Criteria Calculation

As discussed in Section 5, the methods outlined by Stephan et al. (1985) were selected for use in calculating the water quality criteria for protection of aquatic life for molybdenum. This method is heavily dependent upon the four most sensitive genera contained within the toxicity database (Table 7.1) and the number of different genera for which molybdenum toxicity data are available (i.e., sample size).

The four genera determined to be most sensitive to molybdenum toxicity are *Tubifex*, *Euglena*, *Pimephales*, and *Ceriodaphnia* (Table 7.1). The GMAVs and SMAVs for each of these four taxa are the same because toxicity data were available for only one species in each of these genera. Some other genera had more than one species represented in the toxicity database (e.g., *Oncorhynchus*). Therefore, using genus-level toxicity data (GMAVs) rather than species-level data (SMAVs) for calculation of the molybdenum acute criterion reduced the sample size from 17 to 13. Use of a smaller sample size in criterion development acts as a conservative measure and reduces the final criterion value.

Using the selected acute toxicity data and the equations outlined by Stephan et al. (1985), the final criteria maximum concentration (CMC) or acute criterion was calculated to be 6.16 mg Mo/L (Table 7.2). Because insufficient data were available to calculate a chronic criterion independent of the acute criterion, the final ACR value (7.5) was applied to the Final Acute Value (12.3 mg Mo/L) to generate a chronic criterion as per EPA guidance. This calculation resulted in a final chronic criterion of 1.65 mg Mo/L.

As part of this effort, the relationship between the hardness of the test solution and the toxicity of molybdenum was evaluated to determine if a meaningful relationship existed (as it does for several other metals) that might be incorporated into the criteria. No such relationship was observed among the available toxicity and water quality data.

Table 7.1.         Summary of Genus Mean Acute Values (GMAV) calculated for molybdenum based
upon the Species Mean Acute Values (SMAV) determined in Section 3.

	GMAV				
Rank	(mg/L)	Genus	Species	Common Name	
13	10,000.0000	Ictalurus	lctalurus punctatus	channel catfish	
12	7,533.3000	Chironomus	Chironomus tentans	midge	
11	6,790.0000	Lepomis	Lepomis macrochirus	bluegill	
10	3,940.0000	Gammarus	Gammarus fasciatus	scud	
9	2,650.0000	Crangonyx	Crangonyx pseudogracilis	isopod	
8	2,218.0871	Daphnia	Daphnia magna	cladoceran	
7	1,969.7716	Catostomus	Catostomus commersoni	white sucker	
			Catostomus latipinnis	flannelmouth sucker	
6	1,459.6053	Oncorhynchus	Oncorhynchus nerka	kokanee salmon	
			Oncorhynchus mykiss	rainbow trout	
			Oncorhynchus kisutch	coho salmon	
			Oncorhynchus tshawytscha	chinook salmon	
5	1,225.6000	Girardia	Girardia dorotocephala	la flatworm	
4	1,015.0000	Ceriodaphnia	Ceriodaphnia dubia	cladoceran	
3	253.8110	Pimephales	Pimephales promelas	fathead minnow	
2	72.3000	Euglena	Euglena gracilis	protistan	
1	28.9100	Tubifex	Tubifex tubifex	tubificid worm	

**Table 7.2.** Summarized calculations used to determine the acute (CMC) and chronic (CCC) molybdenum criteria based on assembled molybdenum toxicity data.

		GMAV	LN	(LN	P =	
Rank	Genus	(mg/L)	GMAV	$GMAV)^2$	R/(N+1)	$P^{0.5}$
4	Ceriodaphnia	1,015.0000	6.9226	47.9230	0.2857	0.5345
3	Pimephales	253.8110	5.5366	30.6538	0.2143	0.4629
2	Euglena	72.3000	4.2808	18.3255	0.1429	0.3780
1	Tubifex	28.9100	3.3642	11.3178	0.0714	0.2673
		Sum	20.1042	108.2200	0.7143	1.6427

Sample Size (N) = 13

$$S^{2} = \frac{\sum \left( (LN \ GMAV)^{2} \right) - \left( \frac{\left( \sum (LN \ GMAV) \right)^{2}}{4} \right)}{\sum (P) - \left( \frac{\left( \sum (\sqrt{P} \right) \right)^{2}}{4} \right)} = 180.7081$$

$$S = \sqrt{S^{2}} = 13.4428$$

$$L = \frac{\left( \sum (LN \ GMAV) \right) - \left( S \left( \sum (\sqrt{P} \right) \right) \right)}{4} = -0.4944$$

$$A = S \left( \sqrt{0.05} \right) + L = 2.5114$$

$$FAV = e^{A} = 12.3232$$
Criteria Maximum Concentration (CMC) =  $\frac{FAV}{2} = \frac{12.3232}{2} = 6.1616$ 
**CMC = Acute Criterion = 6.16 mg Mo/L**
ACR = 7.5
Final Chronic Value =  $\frac{FAV}{ACR} = \frac{12.3232}{7.5} = 1.6494$ 

Final Chronic Value = Criteria Continuous Concentration (CCC)

CCC = Chronic Criterion = 1.65 mg Mo/L

#### 8.0 Draft Molybdenum Criteria

Based upon the available molybdenum toxicity data which met EPA's quality requirements, the appropriate acute and chronic ambient water quality criteria for protection of aquatic life in Nevada for molybdenum are 6.16 mg/L and 1.65 mg/L, respectively. These values should be applied as total recoverable molybdenum as this was the fraction of molybdenum measured in the toxicity studies upon which these criteria are based.

Because the data used in criteria development were screened to ensure inclusion only of species that occur or are reasonable surrogates for species that occur in Nevada, these criteria are appropriate and expected to be protective of aquatic life when applied as a state-wide water quality standard. Although the data screening process eliminated species that do not occur in Nevada, the only data eliminated from consideration was that for (*Basilichthys australis*) which does not occur in North America.

The draft criteria drived in this report are 4.7 times lower than the corresponding lowest acute value (28.9 mg Mo/L) and 28.8 times lower than the lowest definitive chronic value (47.5 mg Mo/L) available. Additionally, these criteria are much lower than the highest tested concentration shown by McConnell (1977) to have no effects on rainbow trout exposed continuously for one year (17 mg Mo/L). These criteria are therefore likely to be appropriately protective of potential toxicity effects of molybdenum on aquatic life in Nevada (and, likely other areas of the U.S).

As per EPA guidance (Stephan et al. 1985), these criteria should be protective of aquatic life, except possibly when a locally important species is particularly sensitive to molybdenum, if the four-day average concentration of molybdenum does not exceed the chronic criterion of 1.65 mg/L (1,650  $\mu$ g/L) more than once every three years on average and if the one-hour average concentration does not exceed 6.16 mg/L (6,160  $\mu$ g/L) more than once every three years on average and if the one-hour average concentration does not exceed 6.16 mg/L (6,160  $\mu$ g/L) more than once every three years on average and if the one-hour average concentration does not exceed 6.16 mg/L (6,160  $\mu$ g/L) more than once every three years on average.

# 9.0 Bioaccumulation

All of the toxicological studies upon which these draft criteria are based considered direct toxicity effects of molybdenum as related to the concentration of molybdenum in solution. The scientific literature was also reviewed to determine the degree to which molybdenum bioaccumulates and biomagnifies and if bioaccumulation and biomagnification appear to affect the toxicity of molybdenum. Several studies were located that evaluated the bioaccumulation of molybdenum.

Under experimental conditions, Sakaguchi et al. (1981) observed that various species of green algae bioconcentrated molybdenum at ratios between 756 and 2,321 (concentration in algae/concentration in solution). The active uptake of molybdenum by algae is not surprising in that molybdenum is a necessary element involved in nitrogen fixation by algae (Sze 1986). These studies were conducted in a solution with a pH of 5.0 and under various other atypical environmental conditions (e.g., high temperature), which may be one reason these extremely high concentration factors were not observed in other studies.

Benthic macroinvertebrates have been observed to accumulate molybdenum in the vicinity of an abandoned uranium mill and mine site in Utah (Peterson et al., 2002) and Saskatchewan, Canada (Robertson and Liber 2007). Robertson and Liber (2007) observed bioaccumulation and biotasediment accumulation factors for molybdenum in caged *H. azteca* of 2.7 to 24.3 in streams receiving uranium mine and mill effluents.

Short et al. (1971) conducted a field and laboratory experiment which used radiolabeled <sup>99</sup>Mo to study molybdenum accumulation in a lake system. The authors reported a high bioaccumulation factor of 40 for the alga *Nitella flexilis* after 40 days. Much lower bioaccumulation factors were reported for plankton (1.2 after 20 days), Simuliidae (black fly) larvae (21 after 20 days), and Notonectidae (backswimmers, 3.6 after 13 days). Bioaccumulation factors in steelhead trout (*O. mykiss*) were determined to range from 7 in the gastrointestinal tract to 16 in the liver after 13 days exposure. Interestingly, the authors reported that trout exposed to molybdenum in solution, but not allowed to feed did not accumulate molybdenum. This result suggests that trout (and possibly other fish) accumulate molybdenum primarily through food.

Saiki et al. (1993) measured concentrations of molybdenum in the food web (algae, plankton, macroinvertebrates, and fish) of the San Joaquin River, California which receives agricultural drainage with elevated concentrations of molybdenum. This study differed from that of Short et al. (1971) in that it was an observational study of long-term stable molybdenum conditions and resulting effects. These authors determined that molybdenum accumulated in tissues at all levels of the food web, but did not biomagnify (i.e., increase in concentration with increasing trophic level) and were typically lower in invertebrates and fish than in algae. These results support those found by Short et al. (1971) that fish accumulated less molybdenum than algae and invertebrates.

Overall, these studies indicate that molybdenum is rapidly accumulated by aquatic biota. To some degree, this uptake of molybdenum is biologically necessary. It does not appear that

molybdenum biomagnifies, suggesting that biota can regulate non-toxic molybdenum tissue burdens. Based on this review, it does not appear that molybdenum presents additional toxic hazards not accounted for by the criteria based on aquatic toxicological studies. Therefore, the water quality criteria presented above should be considered protective of molybdenum toxicity even though the bioaccumulation of molybdenum by various aquatic biota has been documented.

# 10.0 Field Studies

Two studies were available in the scientific literature in which the effects of elevated molybdenum on aquatic life in receiving systems were evaluated. These studies provide additional insight into the protectiveness of the proposed water quality criteria.

Whiting et al. (1994) evaluated the impacts of discharges from a molybdenum mill and mine on benthic macroinvertebrate communities of receiving streams in British Columbia, Canada. In addition to evaluating communities in streams receiving discharges from the mine operations, streams with no such discharges, but naturally occurring high concentrations of metals as well as reference streams without high metal concentrations were included in this study. These authors observed no impacts to the benthic macroinvertebrate communities at any of these sites relative to conditions in reference sites. Among these monitoring sites was one that was measured to contain mean and maximum molybdenum concentrations (between June 1991 and June 1992) of 24.79 mg/L and 32.5 mg/L, respectively, which are both well in excess of both the acute (6.16 mg/L) and chronic (1.65 mg/L) molybdenum criteria presented here.

In an effort to determine the cause of observed impacts to benthic macroinvertebrate communities exposed to uranium mine and mill effluent in receiving streams in northern Saskatchewan, Canada, Robertson and Liber (2007) conducted in situ bioassays with the amphipod, *Hyalella azteca*. These studies determined that molybdenum concentrations as high as 4.945 mg/L in the surface water and 8.250 mg/L (a value which exceeds both the proposed acute criterion of 6.16 mg/L and the proposed chronic criterion of 1.65 mg/L) in the pore water were not related to observed reductions in the survival of *H. azteca* exposed at each site.

This review did not locate any field studies which identified molybdenum toxicity as a primary causative agent in degraded conditions in an aquatic system. Both studies reviewed here evaluated molybdenum concentrations in excess of the calculated chronic criterion of 1.65 mg/L and one evaluated the effects of a yearly mean concentration of 24.79 mg/L, a value four times higher than the draft acute criterion of 6.16 mg/L. Based on these studies, it appears that the molybdenum water quality criteria presented here should be protective of the aquatic life of Nevada.

## 11.0 Summary

The scientific literature regarding molybdenum toxicity data was reviewed for use in developing a scientifically defensible water quality criteria for molybdenum for protection of aquatic life for the State of Nevada. Assembled data were screened for usage in criteria development based on EPA guidance and on the occurrence of test species in Nevada. Following screening, acute toxicity data were available for 17 species in 13 genera and chronic data were available for five species in five genera. Sufficient acute toxicity data were available to satisfy EPA criteria development methodology (i.e., the "eight family rule"), however there were too few chronic data to develop a chronic criterion independent of the acute criterion. Sufficient data were available to calculate an acute-to-chronic ratio (ACR), which was used to calculate a chronic criterion based on relationships established between the acute and chronic toxicity of molybdenum to four different species.

Based on this work and using EPA methodology, an acute molybdenum criterion of 6.16 mg/L and a chronic molybdenum criterion of 1.65 mg/L were calculated. These values should be applied as total molybdenum concentrations.

Based upon a review of field and laboratory studies, it does not appear that bioaccumulation of molybdenum should be important in establishing molybdenum criteria for the protection of aquatic life. Further, the criteria calculated here are substantially lower than concentrations of molybdenum determined to have no effect on either natural benthic macroinvertebrate communities or in situ test organisms placed in streams receiving mine discharges. Finally, both the acute and chronic criteria values are much lower than concentrations determined to have no effects on rainbow trout after a year of constant exposure.

The criteria presented here were calculated based upon all available, suitable molybdenum data in the scientific literature, appropriate EPA methodology, and should be considered protective of aquatic life in Nevada. Therefore, these criteria are appropriate for adoption as water quality standards for the State of Nevada.

# 12.0 Literature Cited

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# **APPENDIX A – UNUSED STUDIES**

Table A-1. List of studies reviewed, but not used in either derivation of molybdenum water quality criteria or review of molybdenum effects in natural systems and the reasons studies were not used.

Citation	Reasons Not used
Pyle, G.G., S.M. Swanson, and D.M. Lehmkuhl. 2002. Toxicity of uranium mine receiving waters to early life stage fathead minnows (Pimephales promelas) in the laboratory. Environmental Pollution 116: 243-255.	Evaluated toxicity of ambient waters containing mixtures of metals to fathead minnows. No laboratory derived Mo data presented.
Trucco R.G., J. Inda and M.L. Fernandez. 1991. Acute toxicity and accumulation of copper, manganese and molybdenum by Basilichthys australis, In: 17th Annual aquatic toxicity workshop. Vancouver, B.C Can P., Chapman F., Bishay E., Power K., Hall L., Harding D., McLeay M., Nassichuk and W. Knapp Eds, 1132.	Test species native to S. America, no reproducing N. American populations
Jacobs, R. and O. Lind. 1977. The combiend relationship of temperature and molybdenum concentration to nitrogen fixation by Anabaena cylindrica. Microbial Ecology 3: 205-217	Evaluated effects of various concentrations of Mo and Temperature on the nitrogen fixing capabilities of the algae. Study did not present toxicity data, but rather data indicating minimum concentrations of Mo necessary to facilitate nitrogen fixation
Morgan, J.D., D.G. Mitchell, and P.M. Chapman. 1986. Individual and combined toxicity of manganese and molybdenum to mussel, Mytilus edulis, larvae. Bulletin of Environmental Contamination and Toxicology 37: 303-307.	Used marine species tested in saltwater.

# APPENDIX B – ACUTE MOLYBDENUM TOXICITY DATA

Table B-1. Summary of all available acute molybdenum toxicity data considered for use in development of molybdenum water quality criteria.

Species	Common Name	Method	Chemical	Hardness (mg/L as CaCO3)	рН	Test Duration (hr)	Endpoint type	Endpoint Value (mg/L)	Reference
			sodium						
Catostomus commersoni	white sucker	static/renewal	molybdate	111.6	7.9	96	LC50	>2000	Pyle 2000
Catostomus latipinnis	flannelmouth sucker	static	sodium molybdate	144	7.93	96	LC50	1940	Hamilton and Buhl 1997
Calosionus ialipinnis	SUCKEI	Static	sodium	144	7.95	90	LC30	1940	
Ceriodaphnia dubia	cladoceran	static/renewal	molvbdate	86.3	7.19	48	LC50	1015	Canton et al. 2007
			sodium						
Chironomus tentans	midge	static/renewal	molybdate	86	6.58	48	LC50	7533.3	Canton et al. 2007
			sodium						
Crangonyx pseudogracilis	isopod	static	molybdate	50	6.75	96	LC50	2650	Martin and Holdich 1986
			molybdenum			10	1.050		
Daphnia magna	cladoceran	static	oxide	-	8.3	48	LC50	206.8	Kimball 1978
Daphnia magna	cladoceran	static	molybdenum oxide	_	8.3	96	LC50	>430	Kimball 1978
	ciadoceran	314110	sodium		0.0	50	2000	2400	
Daphnia magna	cladoceran	static/renewal	molybdate	86.3	7.19	48	LC50	1727.8	Canton et al. 2007
			sodium						
Daphnia magna	cladoceran	static	molybdate			48	LC50	2847.5	Diamantino et al. 2000
			sodium						
Daphnia magna	cladoceran	static	molybdate	148	7.1	48	TL50	3220	Bionomics 1973
Euglena gracilis	protistan	static	various	-	-	24	NOEC	5.44	Colmano 1973
Euglena gracilis	protistan	static	various	-	-	24	LOEC	960	Colmano 1973
Euglena gracilis	protistan	static	various	-	-	24	Acute	72.3	Colmano 1973
Esox lucius	nothern pike	static/renewal	sodium molybdate	111.6	7.9	96	LC50	>127.7	Pyle 2000
Gammarus fasciatus	scud	static	sodium molybdate	148	7.1	48	TL50	3940	Bionomics 1973
Girardia dorotocephala	flatworm	static/renewal	sodium molybdate	86	6.58	96	LC50	1225.6	Canton et al. 2007
Hyalella azteca	amphipod	static	sodium molybdate	124	8.39	168	LC50	>1.0	Borgmann et al. 2005
			sodium						
Hyalella azteca	amphipod	static	molybdate	18	7.37	168	LC50	>1.0	Borgmann et al. 2005
	a seconda la secol		Mo as AA	101	0.00	400	1.050	0.45	Barrow at al. 2025
Hyalella azteca	amphipod	static	standard	124	8.39	168	LC50	>3.15	Borgmann et al. 2005
Hyalella azteca	amphipod	static	Mo as AA standard	18	7.37	168	LC50	>1.0	Borgmann et al. 2005
lctalurus punctatus	channel catfish	static	sodium molybdate	148	7.1	96	TL50	>10000	Bionomics 1973

### Table B-1. Continued.

Lanamia maaraabirua	bluestill	atatia	sodium molybdate	148	74	96		6700	Dianamiaa 1072
Lepomis macrochirus	bluegill	static	sodium	140	7.1	96	TL50	6790	Bionomics 1973
Morone saxatilis	striped bass	static	molybdate	4430	8.12	96	LC50	>79.8	Dwyer et al. 1992
Oncorhynchus kisutch	coho salmon	static	sodium molybdate	41.7	7.57	96	LC50	> 1000	Hamilton and Buhl 1990
Oncorhynchus mykiss	rainbow trout	static/renewal	sodium molybdate	111.6	7.9	96	LC50	>1190	Pyle 2000
Oncorhynchus mykiss	rainbow trout	static/renewal	sodium molybdate	111.6	7.9	96	LC50	>1000	Pyle 2000
Oncorhynchus mykiss	rainbow trout	static	sodium molybdate	25	6.9	96	LC50	800	McConnell 1977
Oncorhynchus mykiss	rainbow trout	static	sodium molybdate	25	6.9	96	LC50	1320	McConnell 1977
Oncorhynchus mykiss	rainbow trout	static	sodium molybdate	148	7.1	96	TL50	7340	Bionomics 1973
Oncorhynchus mykiss	rainbow trout	static	sodium molybdate	154	7.8	96	TL50	6790	Bionomics 1973
Oncorhynchus mykiss	rainbow trout	static	sodium molybdate	290	7.4	96	TL50	4950	Bionomics 1973
Oncorhynchus nerka	kokanee salmon	static/renewal	sodium molybdate	-	-	96	LC50	>2000	Reid 2002
Oncorhynchus tshawytscha	chinook salmon	static	sodium molybdate	41.7	7.57	96	LC50	> 1000	Hamilton and Buhl 1990
Pimephales promelas	fathead minnow	static/renewal	sodium molybdate	111.6	7.9	96	LC50	>100	Pyle 2000
Pimephales promelas	fathead minnow	static	molybdenum oxide	-	8.56	96	LC50	628	Kimball 1978
Pimephales promelas	fathead minnow	static/renewal	sodium molybdate	85	6.48	96	LC50	644.2	Canton et al. 2007
Pimephales promelas	fathead minnow	-	molybdenum oxide	20	7.4	96	TL50	70	Tarzwell and Henderson 1960
Pimephales promelas	fathead minnow	-	molybdenum oxide	400	8.2	96	TL50	370	Tarzwell and Henderson 1960
Pimephales promelas	fathead minnow	static	sodium molybdate	148	7.1	96	TL50	7630	Bionomics 1973
Tubifex tubifex	tubificid worm	static/renewal	sodium molybdate	245	7.6	96	EC50	28.91	Khangarot 1991

Table B-2. Summary of technical review of each acute molybdenum toxicity value listed in Table B-1 and decision whether to use each value in criteria development.

Species	Common Name	Acute Value (mg/L)	Reference	Used Standard Methods?	Control Performance Acceptable?	Sufficient Documentation to repeat study?	Species Occur in NV?	Exposure Duration appropriate?	Test Solution reasonable?	Decision	Basis for Decision
Catostomus commersoni	white sucker	>2000	Pyle 2000	Yes	Yes	Yes	No	Yes	Yes	Use	
Catostomus latipinnis	flannelmouth sucker	1940	Hamilton and Buhl 1997	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Ceriodaphnia dubia	cladoceran	1015	Canton et al. 2007	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Chironomus tentans	midge	7533.3	Canton et al. 2007	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Crangonyx pseudogracilis	isopod	2650	Martin and Holdich 1986	Yes	Not Reported	Yes	Yes	Yes	Yes	Use	Although control performance was not reported, data are useable as 95% confidence intervals indicated good concentration- response curve and high survival of test organisms at lower concentrations.
Daphnia magna	cladoceran	3220	Bionomics 1973	Yes	Yes	Yes	Yes	Yes	Yes	Not Use	Fed
			Canton et al.								
Daphnia magna	cladoceran	2847.5	2007 Diamantino et al. 2000	Yes	Yes Not Reported	Yes	Yes	Yes	Yes	Use	Although control performance was not reported, data are useable as 95% confidence intervals indicated good concentration- response curve and high survival of test organisms at lower concentrations.
Daprinia magna		2047.3	ai. 2000	162		185	162	165	162	026	No control performance data, unsure of organism
Daphnia magna	cladoceran	206.8	Kimball 1978	Yes	Not Reported	Yes	Yes	Yes	Yes	Not Use	performance

### Table B-2. Continued.

Daphnia magna	cladoceran	>430	Kimball 1978	Yes	Not Reported	Yes	Yes	Yes	Yes	Not Use	Fed
Euglena gracilis	protistan	72.3	Colmano 1973	No	Yes	Yes	Yes	Yes	Yes	Use	
Esox lucius	nothern pike	>127.7	Pyle 2000	Yes	No	Yes	Yes	Yes	Yes	Not Use	15-30% control mortality
Gammarus fasciatus	scud	3940	Bionomics 1973	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Girardia dorotocephala	flatworm	1225.6	Canton et al. 2007	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Hyalella azteca	amphipod	>1.0	Borgmann et al. 2005	No	No	Yes	Yes	No	Yes	Not Use	Used 7-day static non-renewal tests as acute exposures,
Hyalella azteca	amphipod	>1.0	Borgmann et al. 2005	No	No	Yes	Yes	No	Yes	Not Use	while more appropriate methods
Hyalella azteca	amphipod	>3.15	Borgmann et al. 2005	No	No	Yes	Yes	No	Yes	Not Use	would be 96-hr renewal. Only required 80% survival
Hyalella azteca	amphipod	>1.0	Borgmann et al. 2005	No	No	Yes	Yes	No	Yes	Not Use	in controls, while 90% should be required for acute testing. Also, tested concentrations substantially lower than all others included in database and showed no effect.
lctalurus punctatus	channel catfish	>10000	Bionomics 1973	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Lepomis macrochirus	bluegill	6790	Bionomics 1973	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Morone saxatilis	striped bass	>79.8	Dwyer et al. 1992	Yes	Yes	Yes	Yes	Yes	No	Not Use	Highly saline dilution water used in testing
Oncorhynchus kisutch	coho salmon	> 1000	Hamilton and Buhl 1990	Yes	Not Reported	Yes	Yes	Yes	Yes	Use	No effects observed at any Mo concentration, therefore fact that control survival not documented not a concern.
Oncorhynchus mykiss	rainbow trout	7340	Bionomics 1973	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Oncorhynchus mykiss	rainbow trout	6790	Bionomics 1973	Yes	Yes	Yes	Yes	Yes	Yes	Use	

### Table B-2. Continued.

Oncorhynchus			Bionomics								
mykiss	rainbow trout	4950	1973	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Oncorhynchus			McConnell								
mykiss	rainbow trout	800	1977	No	Yes	Yes	Yes	Yes	Yes	Use	
Oncorhynchus	roinhour trout	1220	McConnell 1977	No	Vaa	Vee	Yes	Vaa	Vaa	Llaa	
mykiss	rainbow trout	1320	1977	No	Yes	Yes	res	Yes	Yes	Use	Although control
Oncorhynchus mykiss	rainbow trout	>1190	Pyle 2000	Yes	Not Reported	Yes	Yes	Yes	Yes	Use	no reported mortality in any exposure concentration.
Oncorhynchus mykiss	rainbow trout	>1000	Pyle 2000	Yes	Not Reported	Yes	Yes	Yes	Yes	Use	Although control performance was not reported, data are useable as there was no reported mortality in any exposure concentration.
Oncorhynchus nerka	kokanee salmon	>2000	Reid 2002	Yes	Yes	Yes	Yes	Yes	Yes	Use	correspondence with author confirmed control survival >90%
Oncorhynchus tshawytscha	chinook salmon	> 1000	Hamilton and Buhl 1990	Yes	Not Reported	Yes	Yes	Yes	Yes	Use	No effects observed at any Mo concentration, therefore fact that control survival not documented not a concern.
Pimephales promelas	fathead minnow	7630	Bionomics 1973	Yes	Yes	Yes	Yes	Yes	Yes	Not Use	value more than 10x greater, not use
Pimephales promelas	fathead minnow	644.2	Canton et al. 2007	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Pimephales promelas	fathead minnow	628	Kimball 1978	Yes	Not Reported	Yes	Yes	Yes	Yes	Not Use	No control performance data, unsure of organism performance
Pimephales promelas	fathead minnow	>100	Pyle 2000	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Pimephales promelas	fathead minnow	70	Tarzwell and Henderson 1960	No	Not Reported	No	Yes	Yes	Unknown	Not Use	Insufficient documentation

Table B-2. Continued.

Pimephales promelas	fathead minnow	370	Tarzwell and Henderson 1960	No	Not Reported	No	Yes	Yes	Unknown	Not Use	Insufficient documentation
Tubifex tubifex	tubificid worm	28.91	Khangarot 1991	Yes	Not Reported	Yes	Yes	Yes	Yes	Use	No control survival data, but control organisms were reported to be active

# APPENDIX C – CHRONIC MOLYBDENUM TOXICITY DATA

Table C-1. Summary of all available chronic molybdenum toxicity data considered for use in development of molybdenum water quality criteria.

Species	Common Name	Method	Chemical	Hardness (mg/L as CaCO3)	рН	Test Duration	Test Duration Time Units	Effect	Endpoint type	Endpoint Value (mg/L)	Reference
Carassius auratus	goldfish	static/renewal	sodium molybdate	195	7.4	7	day	Survival	LC50	60	Birge 1978
Catostomus commersoni	white sucker	static/renewal	sodium molybdate	111.6	7.9	12	day	Egg Hatchability	NOEC	1.7	Pyle 2000
Catostomus commersoni	white sucker	static/renewal	sodium molybdate	111.6	7.9	12	day	Egg Hatchability	LOEC	>1.7	Pyle 2000
Catostomus commersoni	white sucker	static/renewal	sodium molybdate	111.6	7.9	12	day	Egg Hatchability	IC25	>1.7	Pyle 2000
Catostomus commersoni	white sucker	static/renewal	sodium molybdate	111.6	7.9	12	day	Time to hatch	NOEC	1.7	Pyle 2000
Catostomus commersoni	white sucker	static/renewal	sodium molybdate	111.6	7.9	12	day	Time to hatch	LOEC	>1.7	Pyle 2000
Catostomus commersoni	white sucker	static/renewal	sodium molybdate	111.6	7.9	12	day	Time to hatch	IC25	>1.7	Pyle 2000
Catostomus commersoni	white sucker	static/renewal	sodium molybdate	111.6	7.9	22	day	Growth	NOEC	1.7	Pyle 2000
Catostomus commersoni	white sucker	static/renewal	sodium molybdate	111.6	7.9	22	day	Growth	LOEC	>1.7	Pyle 2000
Catostomus commersoni	white sucker	static/renewal	sodium molybdate	111.6	7.9	22	day	Growth	IC25	>1.7	Pyle 2000
Ceriodaphnia dubia	cladoceran	static/renewal	sodium molybdate	84	7.87	7	day	Reproduction	EC20	76.9	Canton et al. 2007
Ceriodaphnia dubia	cladoceran	static/renewal	sodium molybdate sodium	84	7.87	7	day	Reproduction	LOEC	76.3	Canton et al. 2007
Ceriodaphnia dubia	cladoceran	static/renewal	sodium molybdate sodium	84	7.87	7	day	Reproduction	NOEC	0	Canton et al. 2007
Ceriodaphnia dubia	cladoceran	static/renewal	molybdate sodium	119.4	7.9	7	day	Reproduction	IC25	47.5	Naddy et al. 1995
Ceriodaphnia dubia	cladoceran	static/renewal	molybdate	119.4	7.9	7	day	Reproduction	IC50	79.7	Naddy et al. 1995
Daphnia magna	cladoceran	static/renewal	molybdenum oxide	-	8.37	28	day	Survival	LC50	0.93	Kimball 1978
Daphnia magna	cladoceran	static/renewal	molybdenum oxide	-	8.37	28	day	Reproduction	LOEC	1.15	Kimball 1978

#### Table C-1. Continued.

Daphnia magna	cladoceran	static/renewal	molybdenum oxide	_	8.37	28	day	Reproduction	NOEC	0.67	Kimball 1978
Daprina magna	Claubceran	static/renewal	sodium	_	0.57	20	uay	Reproduction	NOLC	0.07	Kinbali 1970
Daphnia magna	cladoceran	static/renewal	molybdate	83.1	7.88	21	day	Reproduction	EC20	153.8	Canton et al. 2007
Daprina magna	CIAUUCEIAII	Static/Terlewal	sodium	05.1	7.00	21	uay	Reproduction	L020	155.0	Canton et al. 2007
Daphnia magna	cladoceran	static/renewal	molybdate	83.1	7.88	21	day	Reproduction	LOEC	270.6	Canton et al. 2007
Daprina magna	Claubceran	Static/Terlewal	sodium	05.1	7.00	21	uay	Reproduction	LOLC	270.0	Canton et al. 2007
Daphnia magna	cladoceran	static/renewal	molybdate	83.1	7.88	21	day	Reproduction	NOEC	136.1	Canton et al. 2007
	oladoocram	Statio/Teriewai	sodium	00.1	7.00	21	uuy	Reproduction	NOLO	100.1	Diamantino et al.
Daphnia magna	cladoceran	static/renewal	molybdate	-	_	21	day	Reproduction	EC50	102.1	2000
Baphina magna	oladoocram	Statio/Terrewar	sodium			21	uuy	Reproduction	2000	102.1	Diamantino et al.
Daphnia magna	cladoceran	static/renewal	molybdate	-	-	21	day	Reproduction	LOEC	75	2000
Bapinia magna	oladooolali	otatio/ronowa	sodium			21	uuy	Reproduction	2020		Diamantino et al.
Daphnia magna	cladoceran	static/renewal	molybdate	-	-	21	day	Reproduction	NOEC	50	2000
Dapinia magna	nothern	olalio, fonowar	sodium			21	uuy	Reproduction	11020	00	2000
Esox lucius	pike	static/renewal	molybdate	111.6	7.9	13	day	Growth	NOEC	1.7	Pyle 2000
	nothern		sodium				<i>,</i>				. )
Esox lucius	pike	static/renewal	molybdate	111.6	7.9	13	day	Growth	LOEC	>1.7	Pyle 2000
	nothern		sodium				,				
Esox lucius	pike	static/renewal	molybdate	111.6	7.9	13	day	Growth	IC25	>1.7	Pyle 2000
	narrow-		-								
	mouthed		sodium								
Gastrophryne carolinensis	toad	static/renewal	molybdate	195	7.4	7	day	Survival	LC50	0.96	Birge 1978
	rainbow										
Oncorhynchus mykiss	trout	-	-	25.6	7.05	18	months	Survival	NOEC	18.5	Goettl et al. 1976
	rainbow		sodium								
Oncorhynchus mykiss	trout	static/renewal	molybdate	103	7.7	32	day	Survival	LOEC	400	Davies et al 2005
	rainbow		sodium								
Oncorhynchus mykiss	trout	static/renewal	molybdate	103	7.7	32	day	Survival	NOEC	200	Davies et al 2005
	rainbow		sodium								
Oncorhynchus mykiss	trout	static/renewal	molybdate	103	7.7	32	day	Survival	LC50	>400	Davies et al 2005
	rainbow		sodium								
Oncorhynchus mykiss	trout	static/renewal	molybdate	42	7.85	32	day	Survival	NOEC	750	Davies et al 2005
	rainbow		sodium								
Oncorhynchus mykiss	trout	static/renewal	molybdate	42	7.85	32	day	Survival	LOEC	1000	Davies et al 2005
	rainbow		sodium								
Oncorhynchus mykiss	trout	static/renewal	molybdate	42	7.85	32	day	Survival	LC50	>1500	Davies et al 2005
	rainbow		sodium								
Oncorhynchus mykiss	trout	static/renewal	molybdate	104	7.4	28	day	Survival	LC50	0.73	Birge 1978
	rainbow		sodium		6.9 -						
Oncorhynchus mykiss	trout	static/renewal	molybdate	92 - 110	7.8	28	day	Survival	LC50	0.79	Birge et al. 1980

#### Table C-1. Continued.

Oncorhynchus mykiss	rainbow trout	static/renewal	sodium molvbdate	92 - 110	6.9 - 7.8	28	day	Survival	LC10	0.125	Birge et al. 1980
Cheomynenus mykiss	rainbow	Static/Terie war	sodium	52 110	7.0	20	uay	Guivivai	LOID	0.120	Dirge et al. 1966
Oncorhynchus mykiss	trout	flow through	molybdate	25	6.9	1	vear	Survival	NOEC	17	McConnell 1977
	rainbow	j	sodium				<b>,</b>				
Oncorhynchus mykiss	trout	flow through	molybdate	25	6.9	1	year	Growth	NOEC	17	McConnell 1977
	fathead		sodium					Egg			
Pimephales promelas	minnow	static/renewal	molybdate	111.6	7.9	96	hr	Hatchability	NOEC	100	Pyle 2000
	fathead		sodium					Egg			
Pimephales promelas	minnow	static/renewal	molvbdate	111.6	7.9	96	hr	Hatchability	LOEC	>100	Pvle 2000
	-										. ).0 2000
Pimephales promelas	fathead minnow	static/renewal	sodium molybdate	111.6	7.9	96	hr	Egg Hatchability	IC25	>100	Pyle 2000
	-	Static/Terrewai	1	111.0	1.5	30	111		1023	>100	1 yie 2000
	fathead		sodium	111.0	7.0		1	Time to	NOFO	100	D. I. 0000
Pimephales promelas	minnow	static/renewal	molybdate	111.6	7.9	96	hr	hatch	NOEC	100	Pyle 2000
	fathead		sodium					Time to			
Pimephales promelas	minnow	static/renewal	molybdate	111.6	7.9	96	hr	hatch	LOEC	>100	Pyle 2000
	fathead		sodium								
Pimephales promelas	minnow	static/renewal	molybdate	111.6	7.9	7	day	Growth	IC25	>100	Pyle 2000
	fathead		sodium								
Pimephales promelas	minnow	static/renewal	molybdate	92.6	7.66	28	day	Growth	EC20	163.5	Canton et al. 2007
	fathead		sodium								
Pimephales promelas	minnow	static/renewal	molvbdate	92.6	7.66	28	day	Growth	LOEC	258.4	Canton et al. 2007
	-			02.0	1.00	20	uuy	Cicilia	1010	200.4	Curren of un 2007
Dimonholog promolog	fathead	atatia/ranawal	sodium	02.6	7.66	20	dov	Growth	NOEC	142.0	Conton at al. 2007
Pimephales promelas	minnow	static/renewal	molybdate	92.6	1.66	28	day	Growth	NUEL	143.8	Canton et al. 2007

Table C-2. Summary of technical review of each chronic molybdenum toxicity value listed in Table C-1 and decision whether to use each value in criteria development.

Species	Common Name	Test Duration	Test Duration Time Units	Effect	Endpoint type	Chronic Value (mg/L)	Reference	Used Standard Methodology?	Control Performance Acceptable?	Sufficient Documentation to repeat study?	Species Occur in NV?	Exposure Length appropriate?	Test Solution reasonable?	Decision?	Basis for decision
Carassius auratus	goldfish	7	day	Survival	LC50	60	Birge 1978	No	Not reported	No	Yes	No	Yes	Not Use	Too little documentation, no control data
Catostomus commersoni	white sucker	12	day	Egg Hatchability	NOEC	1.7	Pyle 2000	Yes	Yes	Yes	No	No	Yes	Not Use	Growth endpoint prefered
Catostomus commersoni	white sucker	12	day	Egg Hatchability	LOEC	>1.7	Pyle 2000	Yes	Yes	Yes	No	No	Yes	Not Use	Growth endpoint prefered
Catostomus commersoni	white sucker	12	day	Egg Hatchability	IC25	>1.7	Pyle 2000	Yes	Yes	Yes	No	No	Yes	Not Use	Growth endpoint prefered
Catostomus commersoni	white sucker	12	day	Time to hatch	NOEC	1.7	Pyle 2000	Yes	Yes	Yes	No	No	Yes	Not Use	Growth endpoint prefered
Catostomus commersoni	white sucker	12	day	Time to hatch	LOEC	>1.7	Pyle 2000	Yes	Yes	Yes	No	No	Yes	Not Use	Growth endpoint prefered
Catostomus commersoni	white sucker	12	day	Time to hatch	IC25	>1.7	Pyle 2000	Yes	Yes	Yes	No	No	Yes	Not Use	Growth endpoint prefered
Catostomus commersoni	white sucker	22	day	Growth	NOEC	1.7	Pyle 2000	Yes	Yes	Yes	No	Yes	Yes	Not Use	Point estimate chronic value preferred
Catostomus commersoni	white sucker	22	day	Growth	LOEC	>1.7	Pyle 2000	Yes	Yes	Yes	No	Yes	Yes	Not Use	Point estimate chronic value preferred
Catostomus commersoni	white sucker	22	day	Growth	IC25	>1.7	Pyle 2000	Yes	Yes	Yes	No	Yes	Yes	Use	
Ceriodaphnia dubia	cladoceran	7	day	Reproduction	EC20	76.9	Canton et al. 2007	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Ceriodaphnia dubia	cladoceran	7	day	Reproduction	LOEC	76.3	Canton et al. 2007	Yes	Yes	Yes	Yes	Yes	Yes	Not Use	point estimate, EC20 is better estimate of chronic value
Ceriodaphnia dubia	cladoceran	7	day	Reproduction	NOEC	0	Canton et al. 2007	Yes	Yes	Yes	Yes	Yes	Yes	Not Use	point estimate, EC20 is better estimate of chronic value

Table C-2. Continued.

Ceriodaphnia dubia	cladoceran	7	day	Reproduction	IC25	47.5	Naddy et al. 1995	Yes	Not reported	Yes	Yes	Yes	Yes	Use	Test method cited requires minimum acceptable control performance.
Ceriodaphnia dubia	cladoceran	7	day	Reproduction	IC50	79.7	Naddy et al. 1995	Yes	Not reported	Yes	Yes	Yes	Yes	Not Use	End point not conservative
Daphnia							Canton et al.								conservative
magna	cladoceran	21	day	Reproduction	EC20	153.8	2007	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Daphnia magna	cladoceran	21	day	Reproduction	LOEC	270.6	Canton et al. 2007	Yes	Yes	Yes	Yes	Yes	Yes	Not Use	point estimate, EC20 is better estimate of chronic value
Daphnia magna	cladoceran	21	day	Reproduction	NOEC	136.1	Canton et al. 2007	Yes	Yes	Yes	Yes	Yes	Yes	Not Use	point estimate, EC20 is better estimate of chronic value
Daphnia magna	cladoceran	21	day	Reproduction	EC50	102.1	Diamantino et al. 2000	Yes	Yes	Yes	Yes	Yes	Yes	Not Use	EC50 not a conservative estimate of chronic toxicity
Daphnia magna	cladoceran	21	day	Reproduction	LOEC	75	Diamantino et al. 2000	Yes	Yes	Yes	Yes	Yes	Yes	Use	Use geometric mean of LOEC and NOEC
Daphnia magna	cladoceran	21	day	Reproduction	NOEC	50	Diamantino et al. 2000	Yes	Yes	Yes	Yes	Yes	Yes	Use	Use geometric mean of LOEC and NOEC
Daphnia magna	daphnia	28	day	Survival	LC50	0.93	Kimball 1978	Yes	Yes	Yes	Yes	Yes	Yes	Not use	Measure of survival less sensitive than reproduction, therefore use reproductive endpoints.
Daphnia magna	daphnia	28	day	Reproduction	LOEC	1.15	Kimball 1978	Yes	Yes	Yes	Yes	Yes	Yes	Not Use	Chronic value inexplicably low
Daphnia magna	daphnia	28	day	Reproduction	NOEC	0.67	Kimball 1978	Yes	Yes	Yes	Yes	Yes	Yes	Not Use	Chronic value inexplicably low
Esox lucius	nothern pike	13	day	Growth	NOEC	1.7	Pyle 2000	Yes	Not reported	Yes	Yes	No	Yes	Not Use	Test Duration too short
Esox lucius	nothern pike	13	day	Growth	LOEC	>1.7	Pyle 2000	Yes	Not reported	Yes	Yes	No	Yes	Not Use	Test Duration too short
Esox lucius	nothern pike	13	day	Growth	IC25	>1.7	Pyle 2000	Yes	Not reported	Yes	Yes	No	Yes	Not Use	Test Duration too short
Gastrophryne carolinensis	narrow- mouthed toad	7	day	Survival	LC50	0.96	Birge 1978	No	Not reported	No	No	Yes	Yes	Not Use	Too little documentation, no control data

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Table C-2. Summary

Oncorhynchus mykiss	rainbow trout	28	day	Survival	LC50	0.73	Birge 1978	No	Not reported	Yes	Yes	No	Yes	Not Use	Too little documentation, no control data. Values not confirmed by Davis et al. 2005
Oncorhynchus mykiss	rainbow trout	28	day	Survival	LC50	0.79	Birge et al. 1980	No	Yes	Yes	Yes	No	Yes	Not Use	Values not confirmed by Davis et al. 2005
Oncorhynchus mykiss	rainbow trout	28	day	Survival	LC10	0.125	Birge et al. 1980	No	Yes	Yes	Yes	No	Yes	Not Use	Values not confirmed by Davis et al. 2005
Oncorhynchus mykiss	rainbow trout	32	day	Survival	LOEC	400	Davies et al 2005	Yes	Yes	Yes	Yes	No	Yes	Not Use	methods used inferior and contributed to observed effects
Oncorhynchus mykiss	rainbow trout	32	day	Survival	NOEC	200	Davies et al 2005	Yes	Yes	Yes	Yes	No	Yes	Not Use	methods used inferior and contributed to observed effects
Oncorhynchus mykiss	rainbow trout	32	day	Survival	LC50	>400	Davies et al 2005	Yes	Yes	Yes	Yes	Νο	Yes	Not Use	NOEC and LOEC better measures of actual toxicity than "greater than" value
Oncorhynchus							Davies et al								Although test duration slightly short, only data available and should be
Oncorhynchus	rainbow trout	32	day	Survival	NOEC	750	2005 Davies et al	Yes	Yes	Yes	Yes	No	Yes	Use	used. Although test duration slightly short, only data available and should be
mykiss Oncorhynchus mykiss	rainbow trout	32 32	day day	Survival	LOEC LC50	1000 >1500	2005 Davies et al 2005	Yes	Yes	Yes	Yes	No	Yes	Use Not Use	used. NOEC and LOEC better measures of actual toxicity than "greater than" value

Table C-2. Summary

Oncorhynchus mykiss	rainbow trout	18	months	Survival	NOEC	18.5	Goettl et al. 1976	No	Not reported	No	Yes	Yes	Not reported	Not Use	Insufficient documentation
Oncorhynchus mykiss	rainbow trout	1	year	Survival	NOEC	17	McConnell 1977	No	Yes	Yes	Yes	Yes	Yes	Not Use	Not a definitive value
Oncorhynchus mykiss	rainbow trout	1	year	Growth	NOEC	17	McConnell 1977	No	Yes	Yes	Yes	Yes	Yes	Not Use	Not a definitive value
Pimephales promelas	fathead minnow	28	day	Growth	EC20	163.5	Canton et al. 2007	Yes	Yes	Yes	Yes	Yes	Yes	Use	
Pimephales promelas	fathead minnow	28	day	Growth	LOEC	258.4	Canton et al. 2007	Yes	Yes	Yes	Yes	Yes	Yes	Not Use	point estimate, EC20 is better estimate of chronic value
Pimephales promelas	fathead minnow	28	day	Growth	NOEC	143.8	Canton et al. 2007	Yes	Yes	Yes	Yes	Yes	Yes	Not Use	point estimate, EC20 is better estimate of chronic value
Pimephales promelas	fathead minnow	96	hr	Egg Hatchability	NOEC	100	Pyle 2000	Yes	Yes	Yes	Yes	No	Yes	Not Use	Test Duration too short
Pimephales promelas	fathead minnow	96	hr	Egg Hatchability	LOEC	>100	Pyle 2000	Yes	Yes	Yes	Yes	No	Yes	Not Use	Test Duration too short
Pimephales promelas	fathead minnow	96	hr	Egg Hatchability	IC25	>100	Pyle 2000	Yes	Yes	Yes	Yes	No	Yes	Not Use	Test Duration too short
Pimephales promelas	fathead minnow	96	hr	Time to hatch	NOEC	100	Pyle 2000	Yes	Yes	Yes	Yes	No	Yes	Not Use	Test Duration too short
Pimephales promelas	fathead minnow	96	hr	Time to hatch	LOEC	>100	Pyle 2000	Yes	Yes	Yes	Yes	No	Yes	Not Use	Test Duration too short
Pimephales promelas	fathead minnow	7	day	Growth	IC25	>100	Pyle 2000	Yes	Yes	Yes	Yes	No	Yes	Not Use	Test Duration too short