Acute molybdenum toxicity to rainbow trout and other fish

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Abstract: Molybdenum is generally reported in the literature to be acutely lethal to various fish species at relatively high concentrations (70 to >2000 mg/L). The toxicity of molybdenum to fertilized rainbow trout eggs and alevins (EA), however, is controversial as different bioassays demonstrate a wide toxicity range (0.73 to >90 mg/L Mo). A duplication of a previous study using similar water chemistry along with the standard bioassay protocol demonstrated that molybdenum was not acutely toxic to the early life stages of rainbow trout over 32 d up to a maximum molybdenum concentration of 400 mg/L. An additional bioassay exposing early life stages of rainbow trout to a maximum molybdenum concentration of 1500 mg/L for 32 d did not cause sufficient mortality to allow an LC_{50} to be calculated.

Key words: molybdenum, toxicity, bioassays, rainbow trout.

Résumé : Le molybdène est généralement reconnu dans la littérature comme étant extrêmement toxique pour diverses espèces de poisson à des concentrations relativement élevées (70 à >2000 mg/L). La toxicité du molybdène envers les œufs fertilisés et les alevins de la truite arc-en-ciel prête toutefois à controverse puisque divers essais biologiques montrent une large plage de toxicité (0,73 à >90 mg/L Mo). La duplication d'une étude précédente en utilisant une hydrochimie similaire et le protocole standard d'essai biologique a démontré que le molybdène n'était pas extrêmement toxique pour les premiers stades de vie de la truite arc-en-ciel pendant 32 jours à une concentration maximale de molybdène de 400 mg/L. Un essai biologique additionnel utilisant une méthode standard n'a pu calculer une CL_{50} en exposant les premiers stades de vie de la truite arc-en-ciel à 32 jours d'une concentration maximale de molybdène de 1500 mg/L.

Mots clés : molybdène, toxicité, essais biologiques, truite arc-en-ciel.

[Traduit par la Rédaction]

Introduction

Molybdenum is an important element in the production of alloy steels, lubricants, and chemicals. World production in 2002 and 2003 was approximately 123 000 and 127 000 t, respectively, with Canada producing 7 500 t each year (USGS 2004) mined exclusively in British Columbia (NRC 2003). Water quality investigations near several molybdenum mining sites in British Columbia have found dissolved molybdenum from 0.003 to 0.22 mg/L in background water with a range of 0.005 to 11.4 mg/L at sites downstream of mine discharges (Jones 1999).

As with any mining activity, there is concern regarding the toxicity of mining discharges to aquatic and terrestrial organisms. A search of the USEPA database as well as a search of the primary literature indicates a limited amount of information on the toxicity of molybdenum to aquatic organisms, particularly fish. This has resulted in the development of water quality guidelines that are based on limited data sets.

Most natural waters exhibit conditions (pH > 5) where the principle stable species of molybdenum is generally the molybdate ion (MoO_4^{2-}). Waters with lower pH have either hydrogen molybdate ($HMoO_4^-$) or the cationic MoO_2^+ ion as the predominant form (Jones et al. 1994). Water quality guidelines developed for the protection of aquatic life should consider water quality conditions where the test organisms are generally found using the predominant form of molybdenum. Therefore, molybdate should be the form of investigation to estimate the toxicity of molybdenum in an environmental context. Other forms of molybdenum have been used to test molybdenum toxicity; therefore, toxicity values reported in the literature of various molybdenum forms are summarized in Table 1.

Toxicity tests have demonstrated that molybdenum (added as sodium molybdate) does not appear to be highly toxic to fish (Table 2) with reported LC_{50} concentrations ranging from 70 to over 2000 mg/L Mo of variable test duration using salmonids and other fresh and saltwater fish species. In contrast, two toxicity tests reported that molybdenum could be extremely toxic to developing rainbow trout (*Oncorhynchus mykiss*) reporting two separate 28 d LC_{50} of 0.73 and 0.79 mg/L Mo to embryo/alevins

Received 23 December 2004. Revision accepted 24 June 2005. Published on the NRC Research Press Web site at http://jees.nrc.ca/ on 19 October 2005.

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Written discussion of this article is welcomed and will be received by the Editor until 31 March 2006.

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 doi: 10.1139/S05-021

Table 1. Summary of toxicity studies reporting toxicity of various molybdenum species. All endpoints are 96 h LC_{50}.

Species	Development stage	Molybdenum species	Effect conc. (mg/L Mo)	Water characteristics	Water hardness (mg/L as $CaCO_3$)	Reference
Oncorhynchus mykiss	5.3±0.3 cm		86.6	Dechlorinated domestic water	150–200	IMA (1994)*
Pimephales promelas	Unknown	MoO_3	70	Unknown	20	Tarswell & Hendersen (1960)
	Unknown		370	Unknown	400	
	8–10 mm		628	Well water	Hard	Kimball (1978)
Nemacheilus botia	5.8 cm	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	211	Reconstituted DI	60	Pundir (1989)
Oncorhynchus mykiss	4.6 cm	$(NH_4)_2\ Mo_2O_7$	236	Dechlorinated domestic water	150–200	IMA (1994)*

*IMA. 1994. International Molybdenum Association, London, UK. Unpublished data.

Table 2. Summary of toxicity studies reporting molybdenum toxicity added as sodium molybdate. EA and EAF indicate "embryo/alevin" and "embryo/alevin/fry" tests, respectively.

Species	Development stage	End point	Effect Conc. (mg/L Mo)	Water Characteristics	Water Hardness (mg/L as $CaCO_3$)	Reference
Oncorhynchus mykiss	EA	32 d LC_{50}	>1500	Reconstituted DI	42	This study #2
	EA	32 d LC_{50}	>400	Reconstituted DI	93–113	This study #1
	EA	28 d LC_{50}	0.79	Reconstituted DI	92–110	Birge et al. (1980)
	EA	28 d LC_{50}	0.73	Reconstituted DI	104	Birge (1978)
	EAF	60 d LC_{50}	>30	Spiked site water	301–1290	McDevitt et al. (1999)
	EAF	1 year (growth and mort.)	>17	Dechlorinated domestic water	25	McConnell (1977)
	20 mm	96 h LC_{50}	800			
	55 mm	96 h LC_{50}	1320			
	4.6 cm	96 h LC_{50}	3014	Dechlorinated domestic water	150	IMA (1994)*
	alevin	96 h LC_{50}	>1000	Dechlorinated domestic water	112	Pyle et al. (2001)
Oncorhynchus clarki	EA	30 d LC_{50}	>90	Spiked site water	Unknown	Pickard et al. (1999)
Oncorhynchus kisutch	0.5 g	96 h LC_{50}	>1000	Diluted River water	211	Hamilton & Buhl (1990)
	EAF	20 weeks	>15	River water	9.6	Ennevor (1993)
Oncorhynchus tshawytscha	Eyed egg, alevin, & 0.31 g fry	96 h LC_{50}	>1000	Reconstituted DI	42	Hamilton & Buhl (1990)
	0.5, 0.7 & 1.6 g fry	96 h LC_{50}	>1000	Diluted River water	211	
Oncorhynchus nerka	1–2 g	96 h LC_{50}	>2000	Dechlorinated domestic water	107	Reid (2002)
Basilichthys australis	Unknown	96 h LC_{50}	>50	Unknown	Unknown	Trucco et al. (1990)
	<24 h post fertilization	96 h LC_{50}	>100	Dechlorinated domestic water	112	Pyle et al. (2001)
Esox lucius	<24 h post fertilization	96 h LC_{50}	>128	Dechlorinated domestic water	112	Pyle et al. (2001)
Catostomus commersoni	<24 h post fertilization	96 h LC_{50}	>2000	Dechlorinated domestic water	112	Pyle et al. (2001)
Goldfish (Carassius auratus)	4 d post hatch	7 d LC_{50}	60	Reconstituted DI	195	Birge (1978)

*IMA. 1994. International Molybdenum Association, London, UK. Unpublished data.

(Birge 1978; Birge et al. 1980, respectively) and have been utilized to develop water quality guidelines in Canada, the US, and Argentina (CCME 1999; USDI 1998; República Argentina 2002). The results of these two studies suggest that molybdenum is either highly toxic under specific experimental conditions or these two tests are not representative of molybdenum toxicity. In either case, replication of the experiments is warranted so potential risks associated with discharging molybdenum to the environment can be adequately assessed.

We have replicated the experimental conditions found in Birge (1978) and Birge et al. (1980) following both the methodology and water chemistry to try and resolve the discrepancy between these studies and the majority of the literature. Furthermore, a second embryo–alevin (EA) test was done using currently standard methods to gain further insight into molybdenum toxicity to rainbow trout and provide a better basis for the development of appropriate regulations for molybdenum in the freshwater aquatic environment

Methodology

Two static renewal EA tests were conducted on rainbow trout. The first test used a range of molybdenum concentrations from 0.5 to 400 mg/L while the second test used concentrations from 100 to 1500 mg/L Mo. The first test duplicated the water chemistry and methodology used in Birge (1978) and Birge et al. (1980) using reconstituted de-ionized water, and the second test used softer water (42 mg/L $CaCO_3$ hardness) as specified in the standard test method (Table 3; Environment Canada 1998). The gametes came from the Spring Valley Trout Farm, Langley, B.C. The sperm were checked for motility under a microscope before egg fertilization.

The first test was conducted in 600 mL beakers with the eggs placed on the bottom of the beaker similar to Birge (1978) and Birge et al. (1980) while the second test was conducted in 4 L buckets containing 800 mL open slated plastic beakers to support the eggs and was continually aerated (Environment Canada 1998). Experimental details and differences between the two bioassays are summarized in Table 4. Sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$) was dissolved in exposure water and allowed to sit for 2–12 h prior to each water change during the first test and up to a week for the second test. For both tests, the eggs were exposed to the test water within 1 h of fertilization. The bioassay was conducted for 7 d post 50% hatch in the control vessels. This resulted in a 32 d exposure period for both tests. The dissolved oxygen was checked in the bioassay containers with a YSI 52 dissolved oxygen meter and the temperature and conductivity were monitored with a WTW-LF330 T/C meter that compensates for temperature to give specific conductivity. The pH was measured with a Fisher Scientific Accumet Basic meter standardized with buffers of pH 4, 7, and 10. Sub-samples of bioassay water were submitted to a water quality laboratory for confirmatory molybdenum analysis by ICP spectroscopy. Hardness was calculated from the calcium and magnesium concentrations measured by ICP. A standard toxicant test using zinc sulphate was conducted during both bioassays according to the standard procedures with the results within the two standard deviations of the mean of previous reference tests. The statistical analysis of the data was done using computer software, Toxcalc 5.0, with the Shapiro Wilk's and Bartlet's tests used to confirm a normal distribution in the data. Lethal endpoint values were calculated by the probit method. The NOEC/LOEC values were calculated using Bonferonni *t* Test and Dunnett's test for the first and second tests, respectively.

Results and discussion

Water chemistry

Molybdenum analysis of the prepared bioassay water was within 10% or better of the calculated values for both of the bioassay tests, therefore nominal molybdenum concentrations were used for statistical analysis. Test #1 control water exhibited slight contamination (mean Mo 0.1 $\pm$ 0.06 mg/L), however, as this value is much lower than any observed effects, statistics were done with this as a molybdenum control concentration. There was an extremely large discrepancy between the conductivity reported by Birge et al. (1980) of 176 μS/cm (Table 3) and that measured for the bioassay test #1 of 377 μS/cm given the similar water chemistry. Calculated conductivities using reported conductivity and concentration conversion factors (APHA et al. 1975) gave a specific conductivity (conductivity at 25 °C) of 387 μS/cm for the Birge et al. (1980) test water and 361 μS/cm for the test #1 water used in this bioassay. Birge et al. (1980) reported conductivity that is less than half of the calculated conductivity suggesting an error in either the conductivity measurement or in the reported chemical makeup of the water.

Toxicity

Acceptable control group survival of 81 and 95% for test #1 and #2, respectively, was observed. In both tests, highest concentration exposure groups did not exhibit sufficient mortality to calculate LC_{50} (maximum concentrations 400 and 1500 mg/L Mo). In test #1, there was no significant difference between the mortality of trout of the control groups and groups with molybdenum exposure from 0.5 to 200 mg/L Mo. The lowest observable effect concentration (LOEC) was observed at the highest molybdenum concentration of 400 mg/L Mo. The LOEC in test #2 was observed at 1000 mg/L Mo with 79% survival after 32 d. The groups with the highest molybdenum exposure of 1500 mg/L Mo had an average of 83% survival compared to 95% in the control beakers. Toxicity statistics calculated an LC_{15} and LC_{20} as 365.4 and 1424.8 mg/L Mo for test #1 and test #2, respectively. The better survival in test #2 in comparison to test #1 was most likely attributable to the difference in methodology. In test #1, to duplicate the methodology of Birge et al. (1980), 100 fertilized eggs were placed on the bottom of a 600 mL beaker with water changes occurring twice daily. Some embryos and alevins that died turned

Table 3. Water quality conditions of Birge et al. (1980) and comparative tests from this study.

	Bioassay		
Water quality* parameter	Birge et al. (1980)	Test #1	Test #2
Hardness (mg $L^{-1}CaCO_3$)	92–110	93–113	42
pH	6.9–7.8	7.4–8.0	7.7–8.0
Conductivity (μS/cm)†	176	377	159
Ca	27.1	27.1	7.0
Mg	7.4	7.4	6.0
Na	27.4	27.4	13.1
K	2.6	2.6	1.0
Cl	50.3	50.3	1.0
HCO_3	72.6	72.6	34.9
SO_4	29.2	29.2	39.7
Temperature (°C)	12–13	11.9–13.4	14.0
Dissolved oxygen	9.3–10.1	6.5–10.6	8.3–10.1

*All values in mg/L unless specified, pH has no units.

†The conductivity for test #1 is reported as specific conductivity since the conductivity meter was temperature compensated.

Table 4. Test conditions for bioassays.

Variable	Test #1*	Test #2†
Mo concentration range (mg/LMo)	0/0.5/1.0/10/50/100/200/400	0/100/250/500/750/1000/1500
Container	600 mL beakers	4 L bucket with internal slated beaker
Water renewal	Every 12 h	3 ×/week
Eggs per vessel	100	60
Replicates for each concentration	3–4	4
Photoperiod	14 h light/10 h dark	Dark (<500 lux)
Examination (remove morts)	2 ×/day	3 ×/week

*Experimental procedure to duplicate Birge (1978) and Birge et al. (1980).

†Standard test procedure (Environment Canada 1998).

milky white, and ruptured depositing a sticky substance on the bottom of the test vessel. Live alevins and embryos got caught in this substance and may have suffered from infection, limited oxygen availability, or sustained physical damage as they struggled. This sticky substance was removed during water changes to keep this problem to a minimum; however this likely had a negative effect on fish survival.

It is doubtful that the softer water, as specified in the standard toxicity bioassay (Environment Canada 1998) had a large influence in reducing molybdenum toxicity in test #2. On the contrary, evidence on trace metal toxicity indicates that metals are generally more toxic in water with lower hardness (CCREM 1987; Ministry of Water, Land, and Air Protection 1987).

Summary

The reproduced tests could not replicate the high molybdenum toxicity reported by Birge (1978) or Birge et al. (1980) and are comparable to other studies reporting low acute toxicity of molybdenum to fish. As the toxicity of substances in aquatic environments can be greatly magnified or reduced depending on the water quality characteristics of the receiving waters, it is imperative to take a precautionary approach to interpreting toxicological data and assume all reported data are representative. However, outlier values such as that reported by the Birge studies can have a large influence on water quality guidelines due to limited data sets and need to be re-examined to determine the possibility of it being an experimental artifact in order for guidelines to be developed on sound scientific information. To do this, it is imperative that robust experimental methods are followed and meaningful toxicological endpoints are examined.

Evaluating molybdenum toxicity through acute toxicity studies may not accurately estimate molybdenum toxicity in an environmental context. Reid (2002) reported a loss of equilibrium, increased ventilation and physical stress induced mortality in sockeye salmon at relatively low molybdenum concentrations (25 mg/L). Therefore, novel approaches and sub-lethal endpoints need to be considered in experimental design to accurately assess the potential effects that elevated molybdenum concentrations may have on aquatic ecosystems.

Acknowledgements

Thanks are given to Vizon SciTec Inc. for the use of laboratory space and their bioassay facilities. BC Ministry of Water, Land and Air Protection (Smithers, BC) conducted elemental analysis of the test waters by ICP spectroscopy. The Science Council of British Columbia, Endako Mines, Highland Valley Copper, Inmet Mining Corp., Placer Dome Inc., and Taseko Mines all provided financial support for this research. Comments by two anonymous referees are greatly appreciated.

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