



International Molybdenum Association
Summary of Comments on Proposed Pennsylvania Water
Quality Standards – August 21, 2012

2954

IMO A is a non-profit trade association representing 85% of western world molybdenum production facilities and all western world conversion facilities. IMO A's major activities include intensive efforts to develop scientific data concerning the impact of molybdenum ("Mo") on human health and the environment. IMO A is dedicated to assuring that regulatory standards be based on sound science, and it is in that spirit that IMO A's comments are offered. Those comments are summarized below.

The rationale for moving forward at this time to establish water quality criteria for Mo is, at best, questionable. The database released by PaDEP of alleged "hits" of Mo in Pennsylvania waters in fact contains a mix of instream samples (almost all non-detect) with samples from a few industrial effluent streams – 60% of which were from one facility that has since ceased the chemical process that produced wastewaters bearing molybdenum. In the absence of any national standard for Mo, Pennsylvania is not justified in moving to establish Mo water quality criteria at this time, given that (1) the limited studies upon which PaDEP has attempted to justify its proposal are dated, with more recent studies indicating that a number of the "effects" observations cited could not be replicated by other researchers; and (2) the values proposed by PaDEP are substantially at variance with the most recent scientific data, where recent peer-reviewed and additional ongoing studies suggest that the numbers derived by PaDEP from the early studies cited are not well justified.

The proposed aquatic life water quality criteria do not reflect current best scientific knowledge. PaDEP based its proposal for acute and chronic Mo criteria on selected information for four organisms contained in a database compiled for Nevada, with results that are largely driven by data from one organism (the burrowing worm *T. tubifex*) which was tested under laboratory conditions that imposed unusual stresses – where the organism was placed in beaker water, deprived of its normal substrate and subjected to 96-hours without food. The data relied on by PaDEP would suggest that *T. tubifex* is the most sensitive aquatic species, when in fact this worm is an organism characteristic of strongly-polluted waters. If the questionable *T. tubifex* data are not used, the resulting calculations would be quite different. Indeed, the most recent studies of Mo chronic (non-lethal) effects (*De Schampelaere 2010* and *Heijerick 2012a*) indicate that all freshwater species **chronic** EC₁₀ values exceed PaDEP's proposed **acute** value of 6 mg/L – a result that makes PaDEP's calculated acute value illogical. Further, PaDEP's derivation of a proposed chronic standard based solely on the proposed acute value and application of an acute-to-chronic ratio, ignores the substantial body of chronic effects data now available in the peer-reviewed literature.

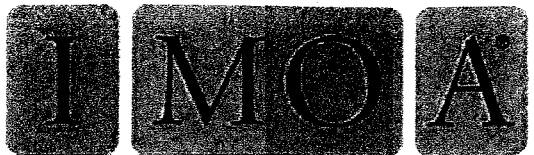
The proposed human health criterion is substantially based upon a single study (*Fungwe 1990*) of female rats, whose data is now subject to serious question as a result of subsequent studies. The *Fungwe* study did not conform to good laboratory practices or modern toxicity study guidelines. As detailed in IMO A's full comments and attachments, more recent studies performed in the United States in accordance with strict OECD toxicity study guidelines, were unable to replicate the *Fungwe* findings even at doses that were much higher than those at which *Fungwe* reported alleged observations of effects. Data from the most recent studies constitute significant weight of evidence leading to the conclusion that the early *Fungwe* study should be disregarded due to its insufficient reliability for regulatory purposes.

In conclusion, in the absence of any pressing problem or need, the prudent course would be to step back for a reexamination of the evolving scientific literature, and then (if necessary) set values that are based on the most recent data.

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**International Molybdenum Association Comments on
Proposed Triennial Review of Water Quality Standards
25 Pa. Code Ch. 93; 42 Pa. Bulletin 4367 (July 7, 2012)
Submitted August 21, 2012**

The International Molybdenum Association ("IMO A") welcomes the opportunity to provide comments to the Pennsylvania Environmental Quality Board ("EQB") and Pennsylvania Department of Environmental Protection ("PaDEP") on the proposed amendments to 25 Pa. Code Chapter 93, concerning establishment of instream water quality criteria as part of the Triennial Review of water quality standards.

IMO A was founded in 1989 as a non-profit trade association with scientific purposes. IMO A represents 85% of western world production facilities and all western world conversion facilities. Among IMO A's major activities is an extensive program lead by the association's health, safety and environmental committee to develop scientific data concerning the impact of molybdenum ("moly" or "Mo") on human health and the environment. In response to the European Union's legislation known as "REACH" (Registration, Evaluation, Authorisation and Restriction of Chemicals), IMO A has established a consortium focused on the pursuit and evaluation of research concerning moly's impacts across the environmental spectrum. It is our hope to share with you as part of these comments the latest information concerning the results of that research and the hazard identification/risk assessment process. IMO A is dedicated to assuring that regulatory standards be based on sound science, and it is in that spirit that these comments are offered.

1. The need for establishing molybdenum water quality criteria is highly questionable.

The rationale for moving forward at this time to establish water quality criteria for Mo is, at best, questionable. The two "rationale" documents submitted by PaDEP to the EQB (*Rationale for the Development of Ambient Water Quality Criteria for the Protection of Human Health* and *Rationale for the Development of Ambient Water Quality Criteria for the Protection of Aquatic Life Use*) contain only cursory and conclusory statements regarding the occurrence of Mo in Pennsylvania waters – stating only that "[b]ecause there is more than one form of molybdenum found in PA waters, the Department is proposing to adopt ... criteria for total molybdenum."¹

¹ IMO A questions PaDEP's claim that "more than one form of molybdenum found in PA waters" for several reasons. First, the speciation of molybdenum in aqueous media as a function of pH and molybdenum concentration has been thoroughly investigated and reported upon in literature. Under physiological conditions (pH > 6.5) the sole molybdenum(VI) species is the molybdate anion, MoO_4^{2-} (Cruywagen, 2000; Cruywagen et al, 2002). Additionally, from an environmental point of view, the significant Mo species is the simple MoO_4^{2-} ion since this is the species which enters the cell in plants and animals (Stiefel, 2002). Molybdenum compounds (e.g., molybdenum trioxide, polymolybdates, ammonium molybdates) transform rapidly to the MoO_4^{2-} ion under environmentally relevant test conditions (Greenwood and Earnshaw, 1997). UV spectra data (Mitchell 2009) also demonstrate that the dissolved molybdenum species is the MoO_4^{2-} ion.

In response to inquiries, the Department released the printout of a data base containing what were characterized as representing the results of more than 400 samples from sites in Pennsylvania, with a purported "median value" of 543 µg/L and a 90th percentile value of 8,264 µg/L. A review of the data base printout, however, reveals that the data set mixes together instream samples (virtually all of which are less than detection levels of 70 µg/L) with what clearly appear to be samples from specific effluent sources. In other words, the mean values calculated by PaDEP do *not* represent instream values, but rather some averaging of stream values and direct effluent samples. Of the 431 samples reported, only 43 contained detectible levels of moly; and of those, more than 60% were from one facility – a facility in north central Pennsylvania that has since ceased the significant chemical process that previously produced molybdenum bearing wastewaters. Other than the samples associated with that one former discharger, the data set indicates that ambient Mo concentrations in Pennsylvania waters are well below the chronic criteria values advocated by the Department to protect aquatic life (values that, as discussed below, we believe are not supported by the latest studies.)

Thus, the question must be posed – why should the Commonwealth press forward with establishing molybdenum criteria at this time? As explained in the following sections, the aquatic and animal toxicity studies upon which the Department has attempted to justify its proposal are dated, with more recent studies indicating that a number of the "effects" observations cited could not be replicated by other researchers. The values calculated by PaDEP are substantially at variance with the most recent scientific data; and recent peer-reviewed and additional ongoing studies suggest that the numbers derived from the early studies cited by PaDEP are not well justified. In the absence of any pressing problem or need, the prudent course would be to step back for a reexamination of the evolving scientific literature, and then (if necessary) set values that are based on the most recent data.

2. The proposed aquatic life water quality criteria for molybdenum do not reflect the current best scientific knowledge.

2.1 PaDEP's approach to deriving aquatic life criteria.

The derivations of PaDEP's proposed instream water quality criteria for Mo are only briefly explained in the document provided to the EQB entitled "*Rationale for the Development of Ambient Water Quality Criteria for the Protection of Aquatic Life Use*" ("**Aquatic Life Rationale Document**"). IMOQA's understanding is that the Department declined to share this document in advance with the Water Resources Advisory Committee, the entity that was created by the Department and has operated for several decades to offer scientific and technical review of such matters. The lack of such critical review by the Department's committee charged with advising on such scientific matters prior to putting forth this proposed rulemaking is regrettable. We note that the Department has indicated that it will open a discussion of these issues in a meeting with the Water Resources Advisory Committee after the close of the public comment period. We would suggest that this discussion not be limited to a single meeting (as apparently is the current plan). The dialogue with stakeholders and experts on the scientific issues discussed below should be open robust and detailed.

PaDEP has proposed an acute ambient water quality criterion 6 mg/L (6000 µg/L) for molybdenum, which it explains was derived based on acute data that were collected from EPA's ECOTOX database and the data presented in the document "Aquatic Life Water Criteria for Molybdenum" that was prepared by TetraTech for the Nevada Division of Environmental Protection in 2008.

PaDEP derived a Final Acute Value ("FAV") is based on the four lowest acute values that were in the Nevada dataset. These consisted of the following listed organisms, with their respective acute LC₅₀ values:

<i>Rank</i>	<i>Species</i>	<i>Common Name</i>	<i>Acute Effect Level (LC₅₀)</i>
1	<i>Tubifex Tubifex</i>	oligochaete	28.9 mg/L
2	<i>Euglena gracilis</i>	protistan	72.3 mg/L
3	<i>Pimephales promelas</i>	fathead minnow	253.8 mg/L
4	<i>Oncorhynchus mykiss</i>	Chinook salmon	1000.0 mg/L

This data set results in a calculated FAV of 12.36 mg/L. PaDEP then applied a safety factor dividing this value by two to arrive at the recommended acute Criterion Maximum Concentration (CMC) to "6.12 (6000 µg/L)."²

The Final Acute Value of 12.36 mg/L also served as starting point for the derivation of a chronic criterion of 1900 µg/L, by applying a calculated Acute-to-Chronic ratio of 6.69 to the Final Acute Value of 12.36 mg/L. Thus, based on acute data only, PaDEP derived an acute ambient water quality criterion (CMC) of 6000 µg/L, and a chronic criterion ("CCC") of 1900 µg/L.

As discussed below, both the Department's Final Acute Value derivation, and the use of the acute-to-chronic ratio methodology to generate a chronic value (while ignoring the latest available chronic effects information) are subject to significant question.

2.2 PaDEP's proposed acute criterion is lower than the chronic water quality values indicated by the most recent peer-reviewed studies.

In field water quality of water quality regulation, the norm would be to expect that acute criteria (those necessary to protect aquatic life from short duration, potentially lethal exposures to chemicals) would be higher than chronic criteria (those designed to protect against adverse effects to long-term exposures). However, in this case, the PaDEP proposed acute value is lower than the chronic water quality values indicated by the most recent peer-reviewed studies. In its Aquatic Life Rationale Document, PaDEP says that it "excluded" consideration of the EURAS (2008) studies (and apparently other chronic effects studies) "due to a lack of acute data" in those studies. It appears that what the Department's staff failed to acknowledge or consider, however, is the relationship between chronic and acute values. One cannot simply ignore available chronic data to derive an alleged "acute" value which is illogically below chronic effects values.

As a result of the data requirements under European Union's REACH legislation, IMOA commissioned an aquatic research program that investigated the chronic toxicity of molybdenum in both freshwater and marine environments. These data have been published in the peer-reviewed journal, *Science of the Total Environment*. Copies of the following publications are appended to these comments:

² It is apparent that the Department's final proposal is a rounded *down* number, which is even more conservative.

- De Schamphelaere KA, Stubblefield W, Rodriguez P, Vleminckx K, Janssen CR, 2010. *The chronic toxicity of molybdate to freshwater organisms I. Generating reliable effects data*. Sci.Total Environ. 408(22), 5362-5371 (attached as **Exhibit A**)
- Heijerick DG, Regoli L, Carey S. 2012a. *The toxicity of molybdate to freshwater and marine organisms. II. Effects assessment of molybdate in the aquatic environment under REACH*. Sci.Total Environ. 435-436, 179-187 (attached as **Exhibit B**)

Table 1 below provides the data for freshwater species from these peer-reviewed papers. Specifically, this table provides the EC₁₀ values, expressed a milligrams per liter of molybdenum. EC₁₀ values represent the highest concentration that will **not have an effect** on 10% of the population of test organisms. In arriving at the EC₁₀ effects value, the evaluated endpoint is not mortality, but a more sensitive endpoint measure that is considered to be ecologically relevant for the evaluation of long-term effects on an organism or its population (for example, growth, growth rate, development, or reproduction). The species listed in this table represent eight different taxonomic groups that are relevant for the freshwater environment.

Table 1: Overview of reliable chronic EC₁₀ values for molybdenum (as molybdate) in the freshwater and marine environment.

Freshwater species	EC ₁₀ (mg Mo/L)
<i>Oncorhynchus mykiss</i> – rainbow trout (De Schamphelaere et al, 2010)	43.2
<i>Pimephales promelas</i> – fathead minnow) (De Schamphelaere et al, 2010; GEI, 2009)	60.2
<i>Ceriodaphnia dubia</i> – water flea (De Schamphelaere et al, 2010; GEI, 2009)	63.0
<i>Pseudokirchneriella subcapitata</i> – green alga (De Schamphelaere et al, 2010)	74.3
<i>Daphnia magna</i> - water flea (De Schamphelaere et al, 2010; GEI, 2009)	89.5
<i>Xenopus laevis</i> – frog (De Schamphelaere et al, 2010)	115.9
<i>Chironomus riparius</i> - midge (De Schamphelaere et al, 2010)	121.4
<i>Brachionus calyciflorus</i> - rotifer (De Schamphelaere et al, 2010)	193.6
<i>Lymnaea stagnalis</i> – snail (De Schamphelaere et al, 2010)	221.3
<i>Lemna minor</i> – duckweed (De Schamphelaere et al, 2010)	241.5

As can be seen from Table 1, all freshwater species **chronic** values well exceed PaDEP's proposed **acute** criterion of 6 mg/L.

It is noteworthy that the two fish species (i.e. *Pimephales promelas* and *Oncorhynchus* sp.) whose acute data were used for PaDEP's derivation of the acute criterion, were the most sensitive freshwater organisms with regard to chronic toxicity.

As part of its research program, IMOA also commissioned a long-term bioaccumulation experiment with the most sensitive species of the chronic data set, i.e. *O. mykiss*. The results of this investigation were recently published in Regoli et al. (2012): Regoli L, Van Tilborg W, Heijerick D, Stubblefield W, Carey S., 2012. *The bioconcentration and bioaccumulation factors for molybdenum in the aquatic environment from natural environmental concentrations up to the toxicity boundary. Sci.Total Environ. 435-436, 96-106* (attached as **Exhibit C**).

In this study, *Oncorhynchus mykiss* (rainbow trout) was exposed for 60 days to varying concentrations of Molybdenum, including 0 (control), 1 and 12 mg Mo/L, followed by a 60-day depuration period. No significant effects on mortality, growth, weight or internal Mo-levels were observed. This indicates that Mo is regulated in the rainbow trout organism (notably the most sensitive species of the data set) up to a value of at least 12 mg Mo/L without causing any adverse observed effects. This value of 12 mg/L represents the freshwater **Predicted No Effect Concentration ("PNEC")** that was derived for molybdenum, using the reliable data that are presented in Table 1.

The point here is clear. PaDEP cannot and should not ignore peer-reviewed chronic effects data for freshwater species in an effort to justify a low acute water quality criterion. Where the chronic EC10 and Predicted No Effects Level values for freshwater species from the latest peer reviewed reports, published in 2010, are *higher* than the PaDEP proposed acute value drawn from an older and more limited data set, something is wrong:

2.3 PaDEP's derivation of the acute aquatic life criterion for Mo is based on toxicity data for a select group of four genus, where one of the genres (an oligochaete / worm) was tested under laboratory conditions which imposed unusual stresses that render the resulting data to be questionable.

As noted above, PaDEP derived its Final Acute Value ("FAV") of 12.36 mg/L based on the four lowest acute values that were in the Nevada dataset, and applied a safety factor to derive a CMC water quality value of 6.12 mg/L, which it rounded to a proposed standard of 6,000 µg/L.

Within the Nevada dataset, acute data were collected for 17 species. Notably, for 14 of these species (82%), the acute value was higher than 1000 mg/L – that is, more than two orders of magnitude higher than the proposed acute criterion of 6 mg/L.

On close examination, PaDEP's derivation of the CMC water quality criteria value was largely driven by the value in the Nevada data for the worm *T. tubifex*. As discussed in the following subsections, use of the *T. tubifex* data requires close examination.

2.3.1 Use of the *T. tubifex* data for deriving a freshwater criterion is questionable.

The low acute criterion derived by PADEP is mainly driven by the value of 28 mg Mo/L that was reported by Khangarot et al (1991) for the oligochaete, *T. tubifex*.

As a starting point, as discussed in greater detail below, *T. tubifex* is a worm that lives its entire life span in stream bottom sediments. It is not a creature that lives in the water column.

Despite this fact, the authors of the Khangarot study exposed field-collected *T. tubifex* to different Mo-concentrations for a 96 hour period in a water-only test design. Under this test

regime, 10 organisms each were exposed to each of the different test concentrations of Mo (administered as sodium molybdate). The test vessels consisted to 200 mL beakers containing 100 mL of test solution. No substrate (natural or artificial sediment) was present in the test vessels. With this setting, the study sought to observe mortality (defined by complete immobilization and no response to pressing with a blunt glass rod).

The authors stated that the worms in the control replicates remained active during the test period: they were clustered at the bottom of the test container and showed typical tubificid movement. In the higher concentration levels, organisms remained separate, showed rapid twining movements and as the test period progressed, there was reduced tactile movement, followed by segmentation/degeneration of the body. At the lethal concentrations, hemoglobin content disappeared and the rear part of the body became white, with disintegration of the body observed.

While the test design followed guidance at the time, and the tests followed the outlined test protocol, there remain major concerns about the relevance and applicability of *T. tubifex* in a water-only exposure test design, as discussed below.

2.3.2 Occurrence and biology of *T. tubifex*

Tubifex tubifex Müller (Annelida: Oligochaeta: Tubificidae), also called sludge worm, sewage worm, or lime snake, is a tubificid oligochaete found in a variety of aquatic habitat types such as the sediments of lakes and rivers (Brinkhurst, 1996; Kaonga et al, 2010). Such worms occur in many aquatic benthic habitats (Reynoldson, 1987); however, substrate is believed to be a primary factor influencing the abundance and distribution of *T. tubifex* (Lazim & Learner, 1987). Fine substrates are favored by *T. tubifex* and are common in stream habitats that have been incised and widened.

T. tubifex worms are “conveyer-belt” deposit feeders, living head-down and partially submerged in the sediment, with the posterior section of their body free in the overlying water to allow cutaneous respiration. Their entire life cycle is spent in full contact with sediment (Chapman et al, 1999). Foraging galleries into the sediment, these worms ingest sediment particles and excrete them at the surface within mucus-bound fecal pellets. This results in intense and ordered mixing of sediment particles, with predominant upward transport that affects solute distribution (e.g. Matisoff, 1995; Pelegri and Blackburn, 1995; Svensson et al., 2001; Mermillod-Blondin et al., 2005; Nogaro et al., 2007).

Notably, *T. tubifex* is also known as species that is characteristic of strongly polluted waters, where it can reach very high densities (Podubnaya, 1980). Milbrink (1987) claimed that *T. tubifex* occurs in these environments where competition or predation is weak (after Dumnicka and Galas, 2002). In oligotrophic situations (conditions characterized by low nutrients), *T. tubifex* is generally a dominating species together *S. ferox* (Milbrink, 1980). The worms can survive with little oxygen through the use of their hemoglobin rich tail-ends, which they wave in order to absorb oxygen. They can also survive in heavily polluted areas with organic matter that almost no other species can endure. *T. tubifex* can also survive drought and food shortage by forming a protective cyst and lowering its metabolic rate (Ward, 1997; Suthar and Sing, 2008).

The tolerance and prevalence of *T. tubifex* in polluted water environments causes a flag when one then examines the purported results of the Khangarot study, which suggests a sensitivity to Mo that is far higher than that of other aquatic species, including a number that are generally

known to be fairly sensitive to other metals. Why would a pollutant tolerant worm be the most sensitive creature in terms of Molybdenum? Is the observed sensitivity real?

2.3.3 Effect of starvation on *T. tubifex*.

As noted above, the Khangarot study utilized a fresh water beaker, devoid of both substrate and food, to test the tolerance of *T. tubifex* to varying concentrations of Mo. The impacts of such stressed conditions must be considered when reviewing the resulting data.

Martinez-Madrid et al (1999) conducted a number of 96 hour starvation experiments with *T. tubifex*. In these experiments, the organisms were exposed to ashed sediments, *i.e.*, sediments that contained no organic material on which the organisms could feed themselves.

Starvation effects were evaluated on somatic growth and reproductive output of worms. Results clearly showed several facts:

- First, there was a quite drastic inhibition of reproduction: only two cocoons were counted in the 3-day-long bioassay, including resorption of clitellum and sperm- and egg-sacs, which suggests that under starvation, the somatic line is preserved in the organism.
- Second, worms lost body biomass during the first days of starvation, although animals were feeding as the gut was seen filled with ashed sediment.
- Third, some worms were seen to fragment in the 3 day starvation bioassay.

In the Martinez-Madrid study, similar observations were not made for *T. tubifex* that were exposed to sediments containing organic material. Clearly, starvation of *T. tubifex* can be a confounding factor in any laboratory evaluation of chemical concentration exposure where the worm is taken out of any substrate and subjected to an extended period without any food.

2.3.4 Relevance of these observations for the data reported by Khangarot (1991).

The findings discussed above must be taken into account when evaluating and considering use of the acute LC₅₀ for molybdenum to the oligochaete *T. tubifex* reported in Khangarot.

T. tubifex is an organism that remains buried head-down in the sediment during its whole life-cycle. Exposing such organisms to test conditions where such burrowing behavior is impossible (glass bottoms of the test vessels do not allow the organism to bury itself) will cause additional stress to the organisms. The clustering of the organisms at the bottom of the test vessel as reported by Khangarot (1991) can be seen as an indicator of stress and an attempt of the organism to be "buried."

The results of the starvation experiment by Martinez-Madrid et al (1999) shows that a relative short period (72 hours) without any food source in sediment already causes significant effects. Starvation even causes fragmentation of some organisms. The worms that were exposed in the tests conducted by Khangarot (1991), were deprived from food for 96 hours (longer than the period that Martinez-Madrid found to induce stress and fragmentation of individual worms). Accordingly, the starvation that was imposed by the Khangarot test protocol could well have contributed to the observation in the Khangarot study of fragmentation / degeneration of the organisms at higher test concentrations. The absence of fragmentation/degeneration at lower

concentration levels might suggest that elevated metal levels contribute to the observed effects, but there is reason to believe that the observed effects are significantly enhanced by the starvation occurring during the test period.

In summary, the presence of a concentration-effect relationship observed in Khangarot indicates that the presence of elevated Mo levels caused adverse effects to *T. tubifex*. However, there are strong indications that the severity of these effects were enhanced by test conditions (absence of substrate and starvation) that induced additional stress. These confounding factors cannot be ignored when reviewing and considering use of the Khangarot data.

Consequently, the reported effect levels for *T. tubifex* likely overestimate the actual toxicity of molybdenum in the aquatic environment. This point is supported by the finding that *T. tubifex* is an organism that is characteristic of strongly polluted waters, where it can reach very high densities (Poddubnaya, 1980, Ward, 1997; Suthar and Sing, 2008). Yet, these field observations are in complete contrast with the reported acute (LC₅₀) toxicity of Mo to *T. tubifex* which is lower than any known chronic No Observed Effects Concentration ("NOEC") or ("EC₁₀") for aquatic freshwater species (see Section 2.2 of this document).

Finally, in the scientific community there is a consensus that assessing risk to benthic species using upper water column organisms is not appropriate (Chapman et al, 1999). Similarly, setting a criterion for the water column should not be based on water-only experiments that are conducted with organisms that spend their whole life-cycle buried in the sediment layer.

2.4 If the questionable *T. tubifex* data is removed from the data set, the calculation of the acute value would be significantly different.

In deriving a Final Acute Value (FAV), the data set should normally contain data for the following freshwater families:

- Salmonidae family (Osteichthyes)
- Second family in Osteichthyes
- Third family in phylum Chordata
- Planktonic crustacean
- Benthic crustacean
- An aquatic insect
- Family in phylum other than Chordata
- Family in any order of insect, or any phylum not already represented

By disregarding the data for *T. tubifex*, the molybdenum data set would not contain a data point for a benthic organism. It may be possible that the value for *T. tubifex* was originally included in the Nevada data set simply in order to fulfill this specific requirement. However, given the questionable value for this organism and taking the significant impact of this data point on the derived water criterion into account, we would respectfully submit that the LC₅₀ for *T. tubifex* should be omitted from the data set.

Recently, however, Liber et al (2011) conducted a series of laboratory spiked-sediment toxicity tests with the benthic amphipod crustacean *Hyalella azteca* and the midge *Chironomus dilutus* to determine acute and chronic toxicity thresholds for molybdenum based on both whole-sediment (total) and pore water exposure concentrations. These results are reported in: Liber K, Doig LE, White-Sobey SL, 2011, *Toxicity of uranium, molybdenum, nickel, and arsenic to*

Hyaella azteca and *Chironomus dilutus* in water-only and spiked-sediment toxicity tests, *Ecotoxicol. Environ. Safe.* 74(5), 1171-1179.

In the Liber study, a number of short-term water-only tests were conducted to evaluate the acute toxicity of molybdenum for these organisms. Molybdenum did not cause mortality of test organisms in either *H. azteca* and *C. dilutus* at any of the concentrations tested. This resulted in the following reported 96-hour LC₅₀ calculations:

- 96 hour LC₅₀ for *H. azteca*: > 741 mg Mo/L
- 96 hour LC₅₀ for *C. dilutus*: > 2,892 mg Mo/L

While some minor effects were noted at the highest test concentrations, the authors commented that such effects were most likely related to elevated sodium levels, and not due to molybdenum exposure.

With this more recent acute data for benthics, continued reliance upon the questionable *T. tubifex* no longer appears justified. If the Department were to set aside the questionable *T. tubifex* data, and instead utilize the acute data for the most sensitive benthic in the Liber study, the crustacean *H. azteca* with an unbounded LC₅₀ greater than 741 mg Mo, the resulting calculation of a Final Acute Value for molybdenum would be 45.29 mg /L. If the FAV is then divided by the safety factor of two, that would result in an acute Criterion Maximum Concentration (CMC) of 22.65 mg Mo/L.

2.5 The proposed chronic water quality criterion for molybdenum rests entirely on the acute value derived using older data, and fails to properly consider available peer-reviewed chronic effects studies.

PaDEP's approach to deriving a chronic water quality criterion is based solely on its calculation of an acute value using older data for four species draft from the Nevada data set, and application of an acute to chronic ratio. PaDEP basically took a calculated acute values for various species, applied an average acute-to-chronic ratio of 6.69 to derive a proposed chronic criterion of 1,900 µg/L. Although PaDEP staff claim that this is based "on the best available toxicological data," that claim is not supported.

As explained above in Section 2.2, published studies that were commissioned by IMO A have generated an extensive chronic dataset for molybdenum for 10 species in the freshwater environment, with chronic EC₁₀ values ranging from 43.2 mg/L to 241.5 mg/L.

It is, therefore, possible to derive directly a Final Chronic Value, based on the four lowest values of EC₁₀ (that is, the values for the four most sensitive fresh water species), without going through the less accurate process of trying to approximate chronic values in various species using an assumed acute-to-chronic ratio.

This exercise of developing a Final Chronic Value based on actual chronic effects data from freshwater species has been published by Heijerick et al (2012): D.G. Heijerick D, Regoli L, Carey S., 2012b. *The toxicity of molybdate to freshwater and marine organisms. II. Effects assessment of molybdate in the aquatic environment under REACH.* *Sci. Total Environ.* 436-436, 179-187. This paper derived a Final Chronic Value of 38.8 mg/L based on all chronic data available (Heijerick et al, 2012b). It should be noted, however, that the U.S. Environmental Protection Agency method does not include data for algal species and higher plants (e.g., green alga, duckweed), and available no-effect levels for these taxonomic groups are generally

discarded when deriving the FCV. If the alga and higher plant data (that is, the data for *P. subcapitata* and *L. minor*) are removed from the Heijerick data, the resulting freshwater FCV becomes 34.8 mg/L.³

3. The proposed human health criterion is substantially based upon a single study whose data is now subject to serious question as a result of subsequent studies.

3.1 The proposed human health criterion is substantially based upon a single study of female rats which did not conform to good laboratory practices or modern toxicity study guidelines.

As explained in the Human Health Rationale Document, PaDEP propose a human health criterion of 0.210 mg/L that is substantially (if not entirely) based upon the results of the Fungwe T.V. *et al* (1990) study referenced in "*Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc*" published by the National Academies Press (2000).

The 9-week Fungwe study on female rats attempted to evaluate the effects of molybdenum doses of within a range measured as milligrams per kilogram of body weight per day ("**mg/kg bw/day**"). Fungwe indicated Mo doses of 0.91 mg Mo/kg bw/day as the tentative No Observed Adverse Effects Level ("NOAEL") and a dose of 1.6 mg/kg bw/day as the tentative Lowest Observed Adverse Effects Level ("LOAEL"). These values were based on a prolonged oestrous cycle, lower gestational weight gain, increased late fetal resorption and embryotoxicity. **Exhibit D** to these comments provides an assessment of why the design and quality of the study are not at all adequate to be used as a developmental toxicity study for regulatory purposes. In short, for a litany of shortcomings, the Fungwe study failed to conform to general requirements of a repeated dose toxicity study (e.g., OECD 415/416 guidelines) or developmental toxicity study guidelines (OECD 414), and as a result the Fungwe study cannot be considered reliable based on current standards for such toxicity tests.

3.2 Subsequent studies conducted under rigorous guidelines have been unable to replicate the Fungwe study results.

In 2011, primarily for the purpose of generating scientifically robust data for the setting of health-based environmental standards in the United States, IMOA commissioned a 90-day oral toxicity study in rats, with extended reproductive toxicity parameters. This Good Laboratory Practices ("GLP") study was performed in the United States, in accordance with OECD Guideline 408 modified to include the additional parameters of estrous cycles and sperm analyses from OECD Guideline 416. Parameters evaluated during the study included viability, clinical observations, ophthalmology, body weights, food consumption, vaginal cytology and estrous cycling, semen quality, clinical pathology (termination of the treatment period), organ weights, macroscopic

³ IMOA notes that the chronic data set currently does not represent a benthic crustacean. IMOA is currently investigating the possibility of conducting a chronic toxicity test with the benthic amphipod *Hyaella azteca*. Generating these data would complete both the acute and chronic data sets with regard to species requirements (replacement of the acute *T. tubifex* value with the acute *H. azteca* value), and would confirm the direct calculation of a Final Chronic Value without the need to use an acute-to-chronic ratio.

observations and microscopic pathology. Dose concentrations were 0, 5, 17 and 60 mg/kg bw/day of Mo (molybdenum in sodium molybdate dihydrate). Thus, the 2011 evaluation used dose levels higher than those looked at in the Fungwe study.

The 2011 study concludes with finding an overall NOAEL of **17 mg Mo/kg bw/day** based on the effects on reduced body weight gains in males and females, and kidney changes in two females seen at 60 mg Mo/kg bw/day. The 2011 study did not find any estrous cycle effects, any testicular (or gonadal) effects or any sperm effects at the tested doses up to 60 mg Mo/kg bw/day. The final report entitled *Sodium Molybdate Dihydrate: a 90-Day Oral Dietary Administration Study in Rats (GLP) Final Report (October 2011)* is attached as **Exhibit E**.

The results of this 2011 OECD guideline-conforming study contrast markedly with the findings reported in Fungwe (1990). Even at doses that were much higher than those at which Fungwe (1990) reported findings, the 2011 study could not replicate the Fungwe observations of rat estrous cycle effects. (Also, no male reproductive effects were observed in the 2011 study). Likewise, it is noteworthy that the study by Howell et al (1993) also did not confirm Fungwe's findings of prolongation of the estrous cycle.

To further investigate the endpoint of prenatal reproductive toxicity that is the key focus of the Fungwe study, IMOIA has commissioned an OECD 414 prenatal development study that is being conducted in the United States by Research Triangle Institute ("RTI") of South Carolina (<http://www.rti.org/>). As of August 2012, RTI has completed the dose range-finding study, and the final report of that step of the evaluation is attached as **Exhibit F**.

In the RTI dose range-finding study, dose concentrations were 0, 1, 5, 10 and 20 mg/kg bw/day of Mo (molybdenum in sodium molybdate dihydrate). Based on Fungwe, one would have expected to see an increase in fetal resorptions, decreased fetal bodyweight and an increase in external malformations in the range-finding evaluations. **But none of these effects were observed.** The range-finding study concluded that "administration of sodium molybdate dihydrate in the diet ad libitum to pregnant rats from [6 days after gestation] to term necropsy on [20 days after gestation] was not associated with any treatment or dose-related maternal findings at any dose or any time during gestation or at scheduled necropsy, including no effects on maternal body weights, weight gains, feed consumption in g/day or g/kg/day, clinical observations, pregnancy indices or organ weights. There were no treatment- or dose-related developmental toxicity findings at any dose, including no effects on pre- or postimplantation loss, fetal numbers, sex ratio, body weights, or fetal external malformations or variations."

The data in the above-described recent studies (provided in Exhibits E and F) constitute significant weight of evidence leading to the conclusion that the data from the earlier Fungwe study (which did not conform to applicable guidelines or good laboratory practices) should be disregarded due to its insufficient reliability for regulatory purposes.

Furthermore, the absence of reproductive toxicity findings in the above-referenced more recent studies is supported by results of the National Toxicological Program's 1997 NTP (TR462) study entitled "Toxicology and Carcinogenesis Study of Molybdenum Trioxide...." The 13-week exposure portion of the NTP study was performed to set the dose level for the two-year portion. The NTP study included a standard toxicology study with haematology, clinical chemistry and necropsy with histopathology examinations including full testes examination, sperm counts and motility examinations. In this inhalation exposure evaluation, rats were exposed to doses of 0, 10, 30 or 100 mg/m³ and mice were exposed to 0, 1, 3, 10, 30 or 100 mg/m³ daily for 13 weeks. Final mean body weights of exposed male and female rats and mice were similar to

those of the control group. There were no significant differences between control and exposed animals in absolute or relative organ weights, haematology or clinical chemistry parameters, sperm counts or motility. The only difference observed was that in rats the liver copper concentrations in treated and controls were the same, but in mice there were significant increases in liver copper concentration after molybdenum trioxide sub-micron particle doses of 30 and 100 mg/m3. However, no chemical treatment-related lesions were observed in either species. The study showed very high absorption systemically, with high blood levels of molybdate. Nevertheless, no evidence of adverse effects on testes or male and female reproductive organs were observed in the NTP study even after two years of administration, or on the testes and sperm counts and motility after 13 weeks administration. IMOIA feels strongly that it is important to take this data into consideration in assessing the possible effects of molybdenum on fertility.

3.3 Basing a human health criterion on the Fungwe study alone is not justified.

For the reasons discussed above, the proposal to move forward with adoption of a human health criterion based on the Fungwe study alone cannot be justified. The Clean Streams Law commands both the Department and EQB to consider "the state of scientific and technological knowledge" (35 P.S. §691.5(a)(3)), and that imposes an obligation to give serious consideration to, and account for, all relevant scientific information. In this case, the best information is represented by the most recent studies that were conducted by reputable laboratories, following rigorous laboratory practices and OECD guidelines – not a 20-year old study that fails to meet modern toxicity study protocols.

4. Conclusion

Again, IMOIA and its members appreciate the opportunity to submit these comments. We support establishment of regulatory standards that are based on sound science. In this particular instance, we believe that a much more critical review of all available scientific studies is required before the Department and Environmental Quality Board move forward. The state of scientific knowledge has moved forward some considerable distance from the time of the studies upon which PaDEP staff have built this proposal. IMOIA stands ready to work with the Department in providing and reviewing the most up to date information so that properly informed, science-based decisions can be made.

Respectfully submitted,



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References:

- Brinkhurst RO, 1996. On the role of tubificid oligochaetes in relation to fish disease with special reference to the myxozoa. *Annual Review of Fish Diseases*, 6, 29–40. Symposium, 26, 167–179.
- Chapman K, Benton M, Brinkhurst R, Scheuerman P, 1999. Use of the aquatic oligochaetes *Lumbriculus variegatus* and *Tubifex tubifex* for assessing the toxicity of copper and cadmium in a spiked-artificial-sediment toxicity test. *Environ.Toxicol.* 14(2), 271–278.
- Cruywagen JJ, 2000. Protonation, oligomerization, and condensation reactions of vanadate(V), molybdate(VI), and tungstate(VI). *Adv. in Inorg. Chem.* 49, 127-182
- Cruywagen JJ, Draaier AG, Heyns JBB, Rohwer EA, 2002. Molybdenum(VI) equilibria in different ionic media. Formation constants and thermodynamic quantities. *Inorg. Chem. Acta* 331, 332-329
- De Schamphelaere KA, Stubblefield W, Rodriguez P, Vleminckx K, Janssen CR, 2010. The chronic toxicity of molybdate to freshwater organisms I. Generating reliable effects data. *Sci.Total Environ.* 408(22), 5362-5371.
- Dumnicka E, Galas J, 2002. Factors affecting the distribution of Oligochaeta in small high mountain ponds (Tatra Mts, Poland). *Arch. Hydrobiol.*, 156(1), 121-133.
- Fungwe T.W. et al (1990). The role of dietary molybdenum on oestrous activity, fertility, reproduction and molybdenum and copper enzyme activities of female rats. *Nutr. Res.* 10, 515-524
- Greenwood NN, Earnshaw A (Eds.), 1997. Chromium, Molybdenum and Tungsten. In: *Chemistry of the Elements*, 2nd Ed., chap. 23, 1002-1039.
- Heijerick DG, Regoli L, Stubblefield W, 2012a. The chronic toxicity of molybdate to marine organisms I. Generating reliable effects data. *Sci.Total Environ.* 430, 260-269.
- Heijerick D, Regoli L, Carey S., 2012b. The toxicity of molybdate to freshwater and marine organisms. II. Effects assessment of molybdate in the aquatic environment under REACH. *Sci.Total Environ.* 436-436, 179-187.
- Howell et al (1993). Effect of thiomolybdate and ammonium molybdate in pregnant guinea pigs and their offspring. *Res Vet Sci* 1993: 55(2):224-230.
- Huntingdon Life Sciences (2011). Final Report on study 10-225, sodium molybdate dihydrate: A 90-day oral dietary administration study in rats (GLP).
- Kaonga C, Kumwenda J, Mapoma H, 2010. Accumulation of lead, cadmium, manganese, copper and zinc by sludge worms; *Tubifex tubifex* in sewage sludge. *Int.J. Environ.Sci.Tech.* 7(1), 119-126.
- Khengarot BS, 1991. Toxicity of metals to a freshwater tubificid worm, *Tubifex tubifex* (Muller). *Bull.Environ.Contamin.Toxicol.* 46, 906-912.

- Lazim MN, Learner MA, 1987. The influence of sediment composition and leaf litter on the distribution of tubificid worms (Oligochaeta). *Oecologia*, 72, 131–136
- Liber K, Doig LE, White-Sobey SL, 2011. Toxicity of uranium, molybdenum, nickel, and arsenic to *Hyalella azteca* and *Chironomus dilutus* in water-only and spiked-sediment toxicity tests. *Ecotoxicol. Environ. Safe.* 74(5), 1171-1179.
- Martinez-Madrid M, Rodriguez P, Perez-Iglesias J, Navarro E, 1999. Sediment toxicity bioassays for assessment of contaminated sites in the Nervion river (Northern Spain). 2. *Tubifex tubifex* reproduction sediment bioassay. *Ecotoxicology* 8, 111-124.
- Matisoff G, 1995. Effects of bioturbation on solute and particle transport in sediments. In: Allen, H.E. (Ed.), *Metal Contaminated Aquatic Sediments*. Ann Arbor Press, Chelsea, MI, USA, pp. 201–253.
- Mermillod-Blondin F, Nogaro G, Datry T, Malard F, Gibert J, 2005. Do tubificid worms influence the fate of organic matter and pollutants in stormwater sediments? *Environ. Pollut.* 134, 57–69.
- Milbrink G, 1980. Oligochaeta communities in pollution biology: the European situation with special reference to lakes in Scandinavia. In: R.O. Brinkhurst, D.G. Cook (Eds.), *Aquatic Oligochaeta Biology*, Plenum Press, New York: 433-455.
- Milbrink G, 1987. Biological characterization of sediments by standardized tubificid bioassays. *Hydrobiologia*, 155: 267-275.
- Mitchell P.C.H. (2009). Speciation of Mo compounds in water. Ultraviolet spectra and REACH read-across report. Unpublished report for the International Molybdenum Association.
- Nogaro G, Mermillod-Blondin F, Montuelle B, Boisson JC, Lafont M, Volat B, Gibert J, 2007. Do tubificid worms influence organic matter processing and fate of pollutants in stormwater sediments deposited at the surface of infiltration systems. *Chemosphere* 70, 315–328.
- NTP (1997a). Toxicology and carcinogenesis studies of molybdenum trioxide (CAS No. 1313-27-5) in F344/Nrats and B6C3F1 mice (inhalation studies). NTP Technical Report 462, NIH Publication No. 97-3378. Testing laboratory: Hazleton Laboratories America Inc (Vienna, VA) and Batelle Pacific Northwest Laboratories (Richland, WA).
- Pelegri SP, Blackburn TH, 1995. Effects of *Tubifex tubifex* (Oligochaeta: Tubificidae) on N-mineralization in freshwater sediments, measured with ¹⁵N isotopes. *Aquat. Microb. Ecol.* 9, 289–294.
- Poddubnaya TL, 1980. Life cycles of mass species of Tubificidae (Oligochaeta). In: R.O. Brinkhurst and D.G. Cook (Eds.), *Aquatic Oligochaeta Biology*. Plenum Press, New York: 175-184.
- Regoli L, Van Tilborg W, Heijerick D, Stubblefield W, Carey S., 2012. The bioconcentration and bioaccumulation factors for molybdenum in the aquatic environment from natural environmental concentrations up to the toxicity boundary. *Sci. Total Environ.* 435-436, 96-106.

- Reynoldson TB, 1987. The role of environmental factors in the ecology of tubificid oligochaetes: an experimental study. *Holarctic Ecology*, 10, 241–248.
- RTI International (2012). Final Study Report on dose range-finding for the developmental toxicity evaluation of sodium molybdate dihydrate (CAS No. 10102-40-6) administered in the diet to CD (Sprague Dawley) rats.
- Stiefel EI, 2002. The biogeochemistry of molybdenum and tungsten. *Metal Ions in Biological Systems* 39, 1-29.
- Suthar S, Singh S, 2008. Vermicomposting of domestic waste by using two epigeic earthworms (*Perionyx excavatus* and *Perionyx sansibaricus*). *Int. J. Environ. Sci. Tech.*, 5 (1), 99-106.
- Svensson JM, Enrich-Prast A, Leonardson L, 2001. Nitrification and denitrification in a eutrophic lake sediment bioturbated by oligochaetes. *Aquat. Microb. Ecol.* 23, 177–186.
- Ward S. 1997. Worms in the aquarium part one: Tubifex. <http://www.gsas.org/Articles/1997/tubifex.html>.

Attachments to the International Molybdenum Association's
Comments can be found on their website at:

<http://www.molybdenumconsortium.org/index.php?id=197>