

From: "James R. Coughlin"
To: "Cynthia Oshita " <COSHITA@oehha.ca.gov>
CC: <jmurray2@sbcglobal.net>
Date: 5/5/2009 4:43 PM
Subject: Comments by IMOA on Molybdenum Trioxide for the CIC
Prioritization Meeting May 29
Attachments: #1011 CTL Ames 2003-IMOA.pdf; #1012 CTL IVMN 2004-IMOA
inc FC.pdf; Scott et 1991.pdf; #0121-Huvinen_2002.pdf;
#1567_Huvinen_1996.pdf; Prop 65_IMOA Comments_May 5 2009.pdf

Dear Cindy,

Attached are comments (in pdf format) being submitted by the International Molybdenum Association (IMOA) on "Molybdenum Trioxide" for the CIC Prioritization Meeting scheduled for May 29. I am also attaching some key references (in pdf format) cited in our comments that were not included in OEHHA's Summary Document on Molybdenum Trioxide, so that they can be made available to CIC members upon request.

If you have any questions or need additional information, please don't hesitate to contact me or Jay Murray, since the IMOA folks are located in the UK.

Thank you for your attention to our submission.

Best regards,

Jim

James R. Coughlin, Ph.D.

President, Coughlin & Associates:

Consultants in Food/Chemical/Environmental Toxicology and Safety
27881 La Paz Road, Suite G, PMB 213
Laguna Niguel, CA 92677

--- Scanned by M+ Guardian Messaging Firewall ---



4 Heathfield Terrace
Chiswick
London
W4 4JE

Tel: 0207 871 1580
Fax: 0208 994 6067
Email: info@imoa.info
Website: www.imoa.info

May 5, 2009

Members of the Carcinogen Identification Committee

Cynthia Oshita
Office of Environmental Health Hazard Assessment
Proposition 65 Implementation
P.O. Box 4010
1001 I Street, 19th floor
Sacramento, California 95812-4010

Re: Prioritization of Molybdenum Trioxide

Dear Chairperson Mack, Members of the Carcinogen Identification Committee, and Ms. Oshita:

On behalf the International Molybdenum Association (**IMO A**), we are writing to recommend that molybdenum trioxide be given a **Low Priority** for further carcinogenicity review. IMO A was founded in 1989 and is registered under Belgian law as a non-profit trade association (ASBL) with scientific purposes. IMO A's current membership of 68 companies represents 85% of Western World production and all conversion facilities; the largest mines in China are also members, together with Chinese converters. Health, Safety & Environment, and Market Development are the core IMO A activities. Along with this submission, we are also providing electronic copies of key references we cite herein that were not included in the OEHHA Summary Document, so that they can be made available to CIC members upon request.

EXECUTIVE SUMMARY

Molybdenum trioxide should be given a Low Priority for the following reasons.

1. **No Authoritative Body has Classified Molybdenum Trioxide as Causing Cancer:** Molybdenum trioxide (MoO_3)(CAS No. 1313-27-5) has not been formally identified as causing cancer by any of Proposition 65's five Authoritative Bodies, including the National Toxicology Program (NTP). Molybdenum trioxide does not meet the "Sufficient Evidence" of carcinogenicity criteria required by an Authoritative Body listing. The NTP has conducted and reported a chronic inhalation carcinogenicity

bioassay of molybdenum trioxide only showing “Some Evidence” of carcinogenicity in the mouse lung. Consequently, the NTP study results do not support an Authoritative Body listing.

2. **Exposure and Uses:** OEHHA noted that exposure to molybdenum trioxide is “limited/occupational.” Actual molybdenum trioxide exposure to the California public/consumers is very limited and insignificant. The majority of molybdenum trioxide sold into California is used in the manufacture of petroleum catalysts by one or two companies. This catalyst is then shipped out of state to be activated, during which process the molybdenum trioxide is converted to molybdenum sulfide. The uses stated by OEHHA actually cover all molybdenum chemicals, not just molybdenum trioxide, and will be separately addressed below.
3. **Animal Carcinogenicity Data (NTP, 1997, 1998):** The key findings of the NTP 2-year bioassay of molybdenum trioxide in rats and mice do not provide “Sufficient Evidence” of a carcinogenic effect:
 - a. Male rats provided only “Equivocal Evidence” of carcinogenicity and female rats provided “No Evidence” of carcinogenicity.
 - b. Only “Some Evidence” of carcinogenicity in the lung was reported by NTP for male and female mice, but these findings did not reach NTP’s highest category of “Clear Evidence.”
 - c. “Biological plausibility” and statistical significance arguments based on studies and criteria published by NTP’s retired Chief of the Biostatistics Branch, Dr. Joseph Haseman, are not satisfied for the carcinogenicity of molybdenum trioxide in the mouse [see Appendix II]:
 - i. Male Mouse Lung Tumors: no statistically significant increase was reached in adenomas at any dose; there was no dose-response in the incidence of carcinomas; the high-dose carcinomas did not reach the required $P < 0.01$ needed for a common tumor; the combined adenomas or carcinomas only reached statistical significance at the required $P < 0.01$ for the low dose.
 - ii. Female Mouse Lung Tumors: adenomas were not statistically significantly increased at the required $P < 0.01$ at any dose; no statistically significant increase was observed in carcinomas at any dose; the combined adenomas or carcinomas only reached statistical significance at the required $P < 0.01$ for the high dose.
 - d. The finely micronized molybdenum trioxide product tested by NTP is not encountered in industrial or consumer exposures. Its micronization by NTP, producing a test substance from 1.3 - 1.5 μm particle size, resulted in over 600 times greater exposure of these particles to the mouse lower lung than if the actual, undensified molybdenum trioxide “Form A” itself had been used in the bioassay.

4. **Animal Carcinogenicity Data (Stoner et al., 1976):** This short-term, high-dose intraperitoneal injection study in mice provides no useful carcinogenicity information on molybdenum trioxide.
5. **Epidemiological Data:** The one epidemiological study claiming to be a positive occupational study of lung cancer (Droste et al., 1999) is based upon an examination of many mixed exposures to various substances, not just to molybdenum trioxide exposure, and is considered to be a poorly conducted study.
6. **Genotoxicity Data:** Molybdenum trioxide is non-genotoxic in the NTP assays and also in three assays conducted for IMO A by the Central Toxicology Laboratory, UK (CTL). Some studies cited by OEHHA that purport to demonstrate positive genotoxicity effects are either studies of molybdenum compounds other than molybdenum trioxide or are deficient because of methodological flaws, particularly when the addition of molybdenum trioxide is known to reduce the pH of the assay systems' culture media and give false-positive effects due to the lowered pH.
7. **Human and Plant Essentiality of Molybdenum:** Molybdenum is an essential trace element with a firmly established RDA for humans (FNB, 2001) and is also essential for other mammals and plants. Molybdenum exposure occurs naturally as an essential nutrient in foods, as an added nutrient in vitamin/mineral supplements (as sodium molybdate, not molybdenum trioxide) and from other molybdate forms, such as sodium molybdate, as a fertilizer in deficient soils. Therefore, it would not serve any California public health interests to list a human and plant essential trace nutrient as a carcinogen under Proposition 65.

1. NO AUTHORITATIVE BODY HAS CLASSIFIED MOLYBDENUM TRIOXIDE AS CAUSING CANCER

Molybdenum trioxide (MoO₃) has not been formally identified as causing cancer by any of Proposition 65's five Authoritative Bodies, including the National Toxicology Program (NTP). OEHHA pointed out in their background prioritization materials released on March 5, 2009 that they applied two data screens (animal and human) to roughly half the chemicals in a tracking database of chemicals to which Californians are potentially exposed. Molybdenum trioxide was one of the chemicals that "passed" OEHHA's animal data screen and was therefore included in the notice requesting public comments.

OEHHA pointed out, however, that "Candidate chemicals that are candidates for listing via an administrative listing mechanism were not screened." We agree with OEHHA that molybdenum trioxide does not qualify as a candidate for Authoritative Body listing, since it does not meet the listing criteria of "Sufficient Evidence" of carcinogenicity required by an Authoritative Body listing (27 CCR Section 25306).

2. EXPOSURE AND USES

Uses of Molybdenum Compounds in California.

As noted by OEHHA in its Summary Table, molybdenum trioxide has only “Limited/occupational” exposure. Molybdenum trioxide does not have widespread industrial use within the State of California, and based upon the limited use of molybdenum trioxide within the State, occupational exposures are expected to be minimal as well. This conclusion is based upon many years of commercial experience by IMOA’s member companies within the State of California. These member companies account for most, if not all, of the molybdenum trioxide transported into the State.

Of the uses for molybdenum trioxide that are cited by OEHHA in the “Molybdenum Trioxide” summary document, the following review for each cited use is provided:

- *“Its major use is as an additive to steel and other corrosion-resistant alloys.”* In fact, though molybdenum trioxide is a component of specialty steels and corrosion resistant alloys, we are not aware of any such production of these alloys in California. For specialty steels that are produced out of state and shipped into California, once the molybdenum trioxide is incorporated into these alloy and specialty steel products, the chemical form of the molybdenum is converted to a metallic alloy and no longer is molybdenum trioxide.
- *“It is also used in the production of molybdenum products.”* In fact, the same analysis presented above for specialty steels and alloys applies to the use of molybdenum trioxide for the production of molybdenum metal products. In this process, molybdenum oxide is reduced by hydrogen in small furnaces to the metal form of molybdenum. The metallic powder can then be converted to other product shapes. We are not aware of any such industrial activities within California. In addition, with molybdenum products shipped into California, the molybdenum trioxide no longer exists, since it was converted to metallic molybdenum.
- *“...as an industrial catalyst”* In fact, the single largest use of pure molybdenum trioxide is as a component in the manufacture of hydrodesulfurization catalysts, and we are only aware of one major company within the State that produces this catalyst. In this manufacturing process, the molybdenum trioxide is incorporated along with other metals into a catalyst matrix such as alumina. Once impregnated into the catalyst, the catalyst is shipped out of state where it is activated at high temperature in a reducing atmosphere, under which conditions the molybdenum trioxide is converted to a sulfide. The final, activated catalyst is then placed into reactor vessels at petroleum refineries to convert sulfur to a gaseous form that can be collected and recovered. Exposures during manufacture of the catalyst are well below state industrial hygiene standards, and exposure once in use in refineries is negligible, since the molybdenum trioxide is no longer present.
- *“a pigment”* Ammonium octamolybdate is the form of molybdenum that is used in the pigment industry. As a result, there will not be any use of or exposure to molybdenum trioxide in this industry.
- *“a crop nutrient”* The chemical form of molybdenum that is used as a crop nutrient supplement (fertilizer) is sodium molybdate and not molybdenum trioxide. This is due to the more neutral pH properties of sodium molybdate. As a

result, there will not be any use of or exposure to molybdenum trioxide as a crop nutrient.

- “*a component of glass, ceramics, and enamels*” It is possible that some form of molybdenum could be used in these applications; however, we are not aware of any such use of molybdenum trioxide for these applications. Furthermore, after firing of the glass, ceramic or enamel, the form of the molybdenum will be converted to a non oxide derivative of molybdenum. Under these circumstances and use, there will not be any exposure to molybdenum trioxide.
- “*a flame retardant*” Ammonium octamolybdate is the chemical form of molybdenum that is used in flame retardant and smoke suppressant applications. Consequently, there will not be any use of or exposure to molybdenum trioxide under this use.
- “*and as a chemical reagent*” IMOA is not aware of any significant uses of molybdenum trioxide as a chemical reagent in California other than as described in the above applications. Therefore, exposures in this area are insignificant.

3. ANIMAL CARCINOGENICITY DATA (NTP, 1997, 1998)

There are three different forms of molybdenum trioxide, and the form tested in the NTP bioassay is not the form to which people are exposed. The NTP published a carcinogenesis bioassay study (NTP Technical Report No. 462, 1997) on molybdenum trioxide in 1997, and the study was subsequently published in the literature (Chan et al., 1998). It was a standard NTP toxicology and carcinogenesis study using F344/N rats and B6C3F1 mice (inhalation studies) of undensified sublimed pure molybdenum trioxide, which has the smallest particle size of the three forms of molybdenum trioxide commercially produced (**see Appendix I, “Form A” of molybdenum trioxide in the table**).

The commercially-produced “Form A” molybdenum trioxide that was provided to NTP for testing had a volume mean diameter of 39 μm , much smaller than the commercially-produced “Form B” (262 μm) or “Form C” (185 μm). However, it is critically important to point out that for the purpose of the two-year inhalation bioassay, the NTP micronized (or air milled) this undensified sublimed pure oxide (“Form A”) to even further reduce the average particle size, ranging from 1.3 - 1.5 μm for the mouse bioassay and 1.5 - 1.7 μm for the rat bioassay. The test product thus obtained and tested was a form of the chemical which is neither commercially available nor would exist during the manufacturing process or during normal handling and use. This “Form A” product is not sold commercially in any significant quantities into California, and California workers and the public cannot possibly be exposed to the further micronized product tested by NTP. In fact, the majority of molybdenum trioxide sold into California is used in the manufacture of petroleum catalysts by one or two companies, during which process the molybdenum trioxide is converted to molybdenum sulfide.

The NTP Study findings throughout the respiratory tract in both rats and mice at termination of the lifetime studies were consistent with exposure to a direct-acting irritant aerosol. This, in turn, is consistent with the low pH (2.4) of the test material in solution, where the resulting acidity during solubilization at the sites of deposition, in association with prolonged

inhalation exposure, would lead to the development of the non-neoplastic responses seen in both species in the nose, larynx and lung. It is well known that larger-sized particles ($> 5 \mu\text{m}$) are deposited primarily in the upper respiratory tract, whereas much finer particles are deposited in the small airways and alveoli. Thus, the very fine, micronized particles ($< 2 \mu\text{m}$) of molybdenum trioxide to which the animals were exposed resulted in over 600 times greater exposure to these particles in the lower lung than if the "Form A" molybdenum trioxide ($39 \mu\text{m}$) had been used in the bioassay.

This evidence indicates that the results of the NTP lifetime study of mice provide no determination of "Clear Evidence" of carcinogenicity. NTP itself actually concluded that there was only "Some Evidence" in the male mouse and female mouse based on the increased incidence of lung tumors, and there was no reported increase of any tumors in the upper respiratory tract. When the patterns and incidences of lung tumors observed in the treated mice are considered in the context of the already high spontaneous background incidence of lung tumors in the B6C3F1 mouse, this further supports that classification of molybdenum trioxide as an animal carcinogen is not scientifically supportable, nor is the listing of molybdenum trioxide as a carcinogen under Proposition 65.

In addition, the NTP reported that the findings in F344/N female rats in the 2-year bioassay provided "No Evidence" of carcinogenicity and the findings in male rats provided only "Equivocal Evidence" of carcinogenicity. NTP termed the male rat's finding of "Equivocal Evidence" as "Uncertain Findings" in the NTP Abstract's Summary Table (page 8), since statistically the increase in lung tumors produced in male rats was only a marginally significant positive trend.

In **Appendix II**, there is a brief background review on the "Statistical and Biological Significance of NTP Cancer Bioassay Findings." This review is based on published articles by Dr. Joseph K. Haseman, who was the NIEHS/NTP Chief of the Biostatistics Branch and Director of Statistical Consulting during his 33-year career there (he retired in 2004). Dr. Haseman was primarily responsible for the experimental design and data analysis of the NTP rodent carcinogenicity program. Many of his papers concerned the statistical design and interpretation of NTP cancer bioassay results, which provide an understanding of how NTP uses statistically significant findings in interpreting its cancer bioassays.

Haseman has written often about a statistical decision rule that closely approximates the scientific judgment process used to evaluate the NTP studies:

"Declare a compound carcinogenic if any common tumor showed a significant ($P < 0.01$) high-dose effect or if a $P < 0.05$ high-dose effect occurred for an uncommon tumor."

Said another way, Haseman's decision rule was described as follows:

"...regard as carcinogenic any chemical that produces a high-dose increase in a common tumor that is statistically significant at the 0.01 level or a high-dose increase in an uncommon tumor that is statistically significant at the 0.05 level."

In essence, the key points made by Haseman were to be aware that it is not appropriate to blindly regard every $P < 0.05$ statistically positive finding as a biological positive, and that when a common tumor is being evaluated, such as the lung tumors seen for molybdenum

trioxide in the B6C3F1 mouse, the finding must reach the $P < 0.01$ level of significance if it is to have any potential biological relevance.

With regard to the NTP Study results for molybdenum trioxide in the male and female mouse lung (see Table 4 in Chan et al., 1998), it is important to point out that some of the specific lung tumor findings not only failed to give an increasing dose-response, but some also did not reach the $P < 0.01$ level of significance required for a common tumor like the mouse lung tumor:

1. Male Mouse Lung Tumors:
 - a. no statistically significant increase was reached in adenomas at any dose;
 - b. there was no dose-response in the incidence of carcinomas;
 - c. the high-dose carcinomas did not reach the required $P < 0.01$ needed for a common tumor;
 - d. the combined adenomas or carcinomas only reached statistical significance at the required $P < 0.01$ for the low dose, but did not at the mid or high dose.

2. Female Mouse Lung Tumors:
 - a. adenomas were not statistically significantly increased at the required $P < 0.01$ at any dose;
 - b. no statistically significant increase was observed in carcinomas at any dose;
 - c. the combined adenomas or carcinomas only reached statistical significance at the required $P < 0.01$ for the high dose.

Given Dr. Haseman's decision rules on both dose-response and statistical significance described above, it is clear that the lung tumor findings for molybdenum trioxide in the NTP male and female mice present very weak evidence to conclude that molybdenum trioxide is a mouse lung carcinogen.

4. ANIMAL CARCINOGENICITY DATA (Stoner et al., 1976)

Stoner et al. (1976) published a short-term, high-dose intraperitoneal injection study of molybdenum trioxide. Groups of 20 strain A mice (10 males and 10 females) received thrice-weekly intraperitoneal (i.p.) injections of 0.85% saline control or 50, 125 or 200 mg molybdenum trioxide/kg bw (total of 19 injections). A single i.p. injection of 20 mg urethane/mouse served as positive control. Mice were sacrificed 30 weeks after the first injection, their lungs were removed and fixed. After 1 to 2 days, the milky-white nodules on the lungs were counted, and a few nodules were examined histopathologically to confirm the typical morphological appearance of adenoma, a benign tumor. Only the highest total dose of 4,750 mg of molybdenum trioxide per kg mouse bw resulted in a statistically significant ($p < 0.05$) increase in the average number of lung adenomas per mouse. No tumors other than lung adenoma were observed. Results of the histological examination of other organs (liver, intestines, thymus, kidney, spleen, salivary, and endocrine glands) were not reported.

The results of this short-term, high-dose, i.p. injection bioassay of molybdenum trioxide do not provide any meaningful or supporting information on the carcinogenicity of the chemical. In addition, this route of exposure is totally irrelevant to assessing the potential carcinogenic risk of molybdenum trioxide to the California public.

5. EPIDEMIOLOGICAL DATA

Droste et al. (1999).

The one epidemiological study claiming to be a positive occupational study of lung cancer (Droste et al., 1999) is based upon an examination of many mixed exposures to various substances, not just to molybdenum trioxide exposure, and is considered to be a poorly conducted study. This study of Belgian male lung cancer patients claims to be the first (and only) study to show an association between occupational exposure to molybdenum (sic) and lung cancer. However, this study is highly methodologically flawed (e.g., exposure was assessed only by self-report and by a job-task exposure matrix) and does not allow any conclusions to be drawn about the potential carcinogenicity of pure molybdenum trioxide. The study involved a hospital-based, case-control investigation with 478 lung cancer cases and 536 controls recruited from 10 hospitals in the Antwerp region. The authors reported that job histories in the categories “manufacturing of transport equipment other than automobiles,” “transport support services” and “manufacturing of metal goods” were significantly associated with lung cancer. When assessed by job-task exposure matrix (JTEM), exposure to molybdenum, mineral oils and chromium were significantly associated with lung cancer risk.

The first source of bias in the paper results from over-selection of the control group. In an effort to “minimize the chance of controls having diseases that may be related to the exposures under study,” the authors drew controls primarily from cardiovascular surgery wards and excluded subjects with “any type of cancer or with any primary lung disease.” The net effect would have been a systematic and significant reduction in the likelihood that any control would have had industrial exposures. This is because exposures to dust and a wide array of industrial chemicals predispose to respiratory irritation and primary lung diseases. It is a fundamental rule of control selection in a case-control investigation that the controls must have the same opportunity for exposure as do the cases. By excluding control subjects with any type of primary lung disease, the authors would have preferentially excluded controls with industrial exposures. If this actually occurred, one would expect the control group to consist of a higher proportion of better educated and wealthier individuals, and that is apparent from the paper. The proportion of controls in the “High” education group exceeds the proportion of cases in that group by 47%, while the proportion of controls in the “High” socioeconomic status group exceeds the proportion of cases in that group by 45%. Exposure to molybdenum may have occurred more frequently among cases because the authors systematically excluded a subset of controls with industrial exposures.

A second set of methodological issues stems from the authors’ method of exposure assessment. They used a combined strategy of directly asking subjects whether they had been exposed to certain potentially carcinogenic substances and then entering the subjects’ reported occupations into a job-exposure matrix. Subjects in job categories with potential carcinogenic exposures were asked additional questions regarding their work tasks in order to generate a job task exposure matrix. Exposures were coded dichotomously (ever or never) as well as by the cumulative duration of exposure years. Since no effort was made to characterize or measure probable dose levels of exposure in the workplace, subjects with trivial exposures over a certain period of years would have been coded identically to individuals with heavy exposures over that same period of years.

In addition, there was apparently no differentiation of dose among various agents for subjects in job or task categories associated with exposure to multiple agents. For example, if a job task entailed heavy exposure to polycyclic aromatic hydrocarbons (PAHs) and asbestos, moderate exposure to arsenic, chromium and nickel, and low-level exposure to molybdenum, the exposure would have been encoded equally for all six. Of the eight job categories considered as entailing exposure to molybdenum, all eight entailed exposure to nickel, seven entailed exposure to arsenic, six entailed exposure to PAHs and six entailed exposure to asbestos. Given the wealth of data implicating those five substances as human lung carcinogens, the implication of molybdenum as a lung carcinogen is likely due to the confounding effect of those other known carcinogenic agents within an exposure matrix lacking dose information. Had the authors' analyses controlled for established industrial lung carcinogens, the molybdenum effect would likely have disappeared. Consequently, this study is not considered to provide any relationship between exposure to molybdenum and cancer.

Huvinen et al. (1996, 2002).

The only high-quality epidemiology study available that specifically addresses workers potentially exposed to molybdenum is a long-term study of ferrochromium and stainless steel manufacturers (Huvinen et al., 1996, 2002). The aim of this study, conducted in Finland, was to determine whether occupational exposure to chromite, trivalent chromium (Cr+3) or hexavalent chromium (Cr+6) caused respiratory diseases, an excess of respiratory symptoms, a decrease in pulmonary function or signs of pneumoconiosis among workers in stainless steel production. Altogether, 203 exposed workers and 81 referents with an average employment of 23 years were investigated on two occasions (in 1993 and 1998). Exposure to total dust and to different chromium species, as well as to other alloying metals (nickel and molybdenum) were monitored regularly and studied separately. The authors reported median air exposure concentrations in the steel melting shop for molybdenum of only 0.0003 mg/m³ and 0.0006 mg/m³ for personal and stationary samples, respectively, an exposure termed "low" by the authors.

The final conclusion of this study was that long term worker exposures (average 23 years) in modern ferrochromium and stainless steel production with low exposures to dusts and fumes containing chromium compounds, nickel and molybdenum did not lead to respiratory system changes detectable by reported symptoms, lung function tests or radiography.

6. GENOTOXICITY DATA

Molybdenum trioxide is non-genotoxic in the NTP assays and also in three assays conducted for IMO by the Central Toxicology Laboratory, UK (CTL, 2004, 2005; attached). Some studies cited by OEHHA that purport to demonstrate positive genotoxicity effects are either studies of molybdenum compounds other than molybdenum trioxide or are deficient because of methodological flaws, particularly when the addition of molybdenum trioxide is known to reduce the pH of the assay systems' culture media and give false-positive effects due to the lowered pH.

NTP (1997).

The 1997 NTP Technical Report included a set of *in vitro* genetic toxicity tests on molybdenum trioxide in five strains of *Salmonella typhimurium* and cytogenetics tests for

chromosomal aberrations and sister chromatid exchanges in cultured Chinese hamster ovary cells. Concentrations of molybdenum trioxide used were 10 - 10,000 µg per plate and all tests were conducted in both the absence and the presence of induced hamster or rat liver S9. Pure molybdenum trioxide did not induce mutations in any of the *S. typhimurium* strains tested (TA97, TA98, TA100, TA1535 and TA 1537) with or without induced hamster or rat liver S9. Negative results were also obtained with molybdenum trioxide in the cytogenetics tests in cultured Chinese hamster ovary cells, and there was no induction of chromosomal aberrations or sister chromatid exchanges with or without S9. The NTP Technical Report concluded that “Molybdenum trioxide was not mutagenic in any of five strains of *Salmonella typhimurium*, and it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells *in vitro*.”

Kerckaert et al., 1996; Gibson et al., 1997.

Positive results for *in vitro* micronucleus and cell transformation assays in Syrian hamster embryo (SHE) cells have been reported for many chemicals, including several metal compounds (Kerckaert et al., 1996; Gibson et al., 1997). Kerckaert et al. (1996) tested five metal chemicals in their SHE cell transformation assay, including molybdenum trioxide, cobalt sulfate hydrate, gallium arsenide, nickel (II) sulfate heptahydrate and vanadium pentoxide. The cobalt, gallium and vanadium compounds yielded significant morphological transformations at multiple doses of less than 1 µg/mL, the nickel sulfate required a dose of 5 µg/mL, but molybdenum trioxide required a dose of at least 75 µg/mL to yield significant morphological transformations, making it the weakest potency chemical among these other metals.

Gibson et al. (1997), on the other hand, tested 16 organic chemicals and metal compounds being tested at the time in NTP carcinogenicity studies in their *in vitro* SHE cell micronucleus assay. The main purpose of their study was to examine the overall concordance between induction of SHE cell micronuclei and other reports on transformation of SHE cells. They reported that molybdenum trioxide tested positive in their assay.

However, it is very likely that the results of these two studies of molybdenum trioxide are considered to be attributable to a significant reduction in the pH of the incubation media that is known to occur as a result of solubilization of molybdenum trioxide. There was evidence that dissolution of the molybdenum trioxide was associated with a significant reduction of pH in both water and in the incubation media used in these tests. It is well acknowledged that a reduction in pH in *in vitro* mammalian cell assays can result in false positive results. A report from the International Commission for the Protection Against Environmental Mutagens and Carcinogens (ICPEMC) recommended that “...positive results associated with pH shifts in the test system of greater than 1 unit should be viewed with caution and confirmed in experiments conducted at neutral pH” (Scott et al., 1991).

Titenko-Holland et al. (1998).

This study reported data from three assays: (1) an *in vitro* micronucleus assay of two molybdenum salts, ammonium molybdate and sodium molybdate, in human lymphocytes; (2) an *in vivo* mouse micronucleus assay of sodium molybdate in mouse bone marrow; and (3) a preliminary investigation using the *in vivo* mouse dominant lethal assay of sodium molybdate. The chemical formulas of ammonium molybdate and sodium molybdate differ significantly from molybdenum trioxide [MoO₃]:



The authors summarized their results as yielding “moderately positive results at relatively high doses in three experimental systems.” However, IMO A considers that there are substantial weaknesses and flaws in the conduct of these studies. In addition, such studies should be conducted with molybdenum trioxide, since molybdenum trioxide’s solubility characteristics are considerably different from other molybdate salts, and the products of reaction with biological fluids *in vivo* are unknown. Consequently, the studies reported by Titenko-Holland et al. (1998) provide no evidence for any *in vitro* or *in vivo* genotoxicity of molybdenum trioxide itself.

Central Toxicology Laboratory (2004, 2005).

In unpublished studies conducted by the Central Toxicology Laboratory, UK (CTL, 2004, 2005, attached), the IMO A commissioned studies on undensified sublimed pure molybdenum trioxide (Form A). This substance was tested in four strains of *S. typhimurium* (TA 98, TA 100, TA 1535 and TA 1537) and in *Escherichia coli* WP2P uvrA, in accordance with OECD Test Guideline 471. In order to evaluate the substance’s clastogenic and aneugenic potential, it was also tested in an *in vitro* micronucleus assay using human lymphocytes in which the pH of the medium was adjusted to maintain the normal pH of the assay. In both assays, the compound was tested over a range of concentrations, both in the presence and absence of an induced rat liver-derived metabolic system (S9-mix). The bacterial tests were conducted in duplicate and the micronucleus test in triplicate. It was concluded that, under the conditions of the assays in bacteria, the “Form A” test substance, at concentrations from 100 - 5000 µg per plate, gave a non-mutagenic response in the tested strains of *S. typhimurium* and *E. coli* in both the presence and absence of metabolic activation, while positive control substances gave the expected responses. Furthermore, in the micronucleus assay, cytotoxicity was assessed by the use of binucleate index and genotoxicity was assessed by the incidence of micronucleated binucleate cells. “Form A” molybdenum trioxide also proved negative in this assay.

Genotoxicity Summary and Conclusions.

Pure molybdenum trioxide was found not to have any genotoxic activity in a series of well-conducted *in vitro* studies by both NTP and CTL. The positive *in vitro* micronucleus and cell transformation assays in SHE cells reported for molybdenum trioxide by Kerckaert et al. (1996) and Gibson et al. (1997) are considered to be flawed as evaluations of molybdenum trioxide, because there were artifacts arising from the reduced pH of the culture medium following dissolution of the molybdenum trioxide. It is concluded, therefore, that pure molybdenum trioxide tested at the proper pH is not genotoxic in these *in vitro* or *in vivo* assays, and that the positive assay results using ammonium molybdate and sodium molybdate should not be used to assess the genotoxicity of molybdenum trioxide.

7. HUMAN AND PLANT ESSENTIALITY OF MOLYBDENUM

Molybdenum is well known to be an essential trace mineral for humans, animals and plants (Food and Nutrition Board, FNB, 2001; Turnlund et al., 1995) involving several enzymes important to metabolism: mammalian xanthine oxidase/xanthine dehydrogenase, aldehyde

oxidase, sulfite oxidase, formate dehydrogenase, nitrate reductase and nitrogenase. It is also essential for plant production, even though present in plant tissue at a level much lower (0.5 ppm dry matter basis) than the critical levels for other essential elements. Molybdenum is needed for at least three human enzymes: (1) sulfite oxidase catalyses the oxidation of sulfite to sulfate, necessary for metabolism of sulfur amino acids, and sulfite oxidase deficiency or absence leads to neurological symptoms and early death; (2) xanthine oxidase catalyses oxidative hydroxylation of purines and pyridines including conversion of hypoxanthine to xanthine and xanthine to uric acid; and (3) aldehyde oxidase oxidizes purines, pyrimidines, pteridines and is involved in nicotinic acid metabolism. Low dietary molybdenum leads to low urinary and serum uric acid concentrations and excessive xanthine excretion.

The **Recommended Dietary Allowance (RDA)** for molybdenum for adult men and women is 45 µg/day. The average dietary intake of molybdenum (determined by the FNB) by adult men and women is 109 and 79 µg/day, respectively, and the median intake from supplements (determined by the Third National Health and Examination Survey) is about 23 and 24 µg/day for men and women who took supplements, respectively. It is known that the molybdenum content of plant foods varies depending upon the soil content in which they are grown, with legumes being the major contributors of dietary molybdenum, as well as grain products and nuts. Animal products, fruits and many vegetables are generally low in molybdenum. In addition, dietary supplements contain molybdenum in the form of added sodium molybdate, but molybdenum trioxide is not used in vitamin/mineral supplements.

Molybdenum also has several essential functions in plant growth and is required in a constant and continuous supply for normal assimilation of nitrogen. In this regard, it is a component of the enzyme nitrogenase, which is required in nitrogen fixation; legumes fix nitrogen, require more of it than cereals and thus are more sensitive to low molybdenum levels in soil. Sodium molybdate and ammonium molybdate are the molybdenum fertilizer materials most commonly used.

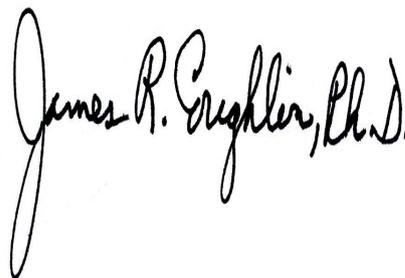
CONCLUSION

In conclusion, molybdenum trioxide should be given a Low Priority.

Sincerely yours,



Sandra Carey
HSE Executive
International Molybdenum
Association
sandracarey@imoa.info
Tel: +44 (0) 7778 813721



James R. Coughlin, Ph.D.
President, Coughlin & Associates
27881 La Paz Rd., Suite G, PMB 213
Laguna Niguel, CA 92677
jrcoughlin@cox.net
Tel: 949-916-6217

[Approved but not signed]

F. Jay Murray, Ph.D.

President, Murray & Associates

5529 Perugia Circle

San Jose, CA 95138

jmurray2@sbcglobal.net

Tel: 408-239-0669

REFERENCES

Central Toxicology Laboratory. 2004. Sublimed Undensified Molybdenum Trioxide: Bacterial Mutation Assay in *S. Typhimurium* & *E. Coli*. Alderley Park, UK, April.

Central Toxicology Laboratory. 2005. Sublimed Undensified Molybdenum Trioxide: In-vitro Micronucleus Assay in Human Lymphocytes. Alderley Park, UK, May.

Chan PC, Herbert RA, Roycroft JH, Haseman JK, Grumbein SL, Miller RA, Chou BJ. 1998. Lung tumor induction by inhalation exposure to molybdenum trioxide in rats and mice. *Toxicol. Sci.* 45: 58-65.

Dixon D, Herbert RA, Kissling GE, Brix AE, Miller RA and Maronpot RR. 2008. Summary of chemically induced pulmonary lesions in the National Toxicology Program (NTP) Toxicology and Carcinogenesis Studies. *Toxicol. Pathol.* 36: 428-439.

Droste JH, Weyler JJ, Van Meerbeeck JP, Vermeire PA, van Sprundel MP. 1999. Occupational risk factors of lung cancer: a hospital based case-control study. *Occup. Environ. Med.* 56: 322-7.

Food and Nutrition Board. 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Chapter 11, Molybdenum, pp. 420-441. Institute of Medicine, National Academy of Sciences, National Academy Press, Washington, DC.

Gibson DP, Brauninger R, Shaffi HS, Kerckaert GA, LeBoeuf RA, Isfort RJ, Aardema MJ. 1997. Induction of micronuclei in Syrian hamster embryo cells: comparison to results in the SHE cell transformation assay for National Toxicology Program test chemicals. *Mutat. Res.* 392: 61-70.

Haseman JK. 1983. Statistical support of the proposed National Toxicology Program protocol. *Toxicol. Pathol.* 1: 77-82.

- Haseman JK. 1984. Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* 58: 385-392.
- Haseman JK. 1995. Data analysis: Statistical analysis and use of historical control data. *Regul. Toxicol. Pharmacol.* 21: 52-59.
- Haseman JK and Elwell MR. 1996. Evaluation of false positive and false negative outcomes in NTP long-term rodent carcinogenicity studies. *Risk Analysis* 16: 813-820.
- Huvinen M, Uitti J, Zitting A, Roto P, Virkola K, Kuikka P, Laippala P, Aitio A. 1996. Respiratory health of workers exposed to low levels of chromium in stainless steel production. *Occup. Environ. Med.* 53: 741-747.
- Huvinen M, Uitti J, Oksa P, Palmroos P, Laippala P. 2002. Respiratory health effects of long-term exposure to different chromium species in stainless steel production. *Occup. Med. (Lond.)* 52: 203-212.
- Kerckaert, GA, LeBoeuf, RA, Isfort, RJ. 1996. Use of the Syrian hamster embryo cell transformation assay for determining the carcinogenic potential of heavy metal compounds. *Fundam. Appl. Toxicol.* 34: 67-72.
- National Toxicology Program (NTP, 1997). *Toxicology and carcinogenesis studies of Molybdenum Trioxide (CAS No. 1313-27-5) in F344/N rats and B6C3F1 mice (Inhalation Studies)*. Technical Report No. 462, Research Triangle Park, NC.
- Scott D, Galloway SM, Marshall RR, Ishidate M, Brusick D, Ashby J, Myhr BC. 1991. Genotoxicity under extreme culture conditions: A Report from ICPEMC Task Group 9. *Mutat. Res.* 257: 147-204; cited at page 200.
- Stoner GD, Shimkin MB, Troxell MC, Thompson TL, Terry LS. 1976. Test for carcinogenicity of metallic compounds by the pulmonary tumor response in strain A mice. *Cancer Res.* 36: 1744-1747.
- Titenko-Holland N, Shao J, Zhang L, Xi L, Ngo H, Shang N, Smith MT. 1998. Studies on the genotoxicity of molybdenum salts in human cells in vitro and in mice in vivo. *Environ. Mol. Mutagen.* 32: 251-259.
- Turnlund JR, Keyes WR, Peiffer GL, Chiang G. 1995. Molybdenum absorption, excretion, and retention studied with stable isotopes in young men during depletion and repletion. *Am. J. Clin. Nutr.* 61: 1102-1109.

APPENDIX I: Chemistry of Molybdenum Trioxide and Chemical Analysis of Molybdenum Compounds.

Physical Form of Molybdenum Trioxide (MoO₃) Tested in the NTP Bioassay.

Molybdenum trioxide is commercially produced in three forms, only one of which (**“Form A” bolded in the following table**) was tested in the NTP 2-year chronic carcinogenicity inhalation bioassay.

	<i>Form A</i>	<i>Form B</i>	<i>Form C</i>
	NTP Studies 2-year inhalation	NTP did not study	NTP 14-day & 13-week studies
Material description Mo trioxide	Undensified sublimed pure	Densified sublimed pure	Chemically produced pure
CAS No.	1313-27-5	1313-27-5	1313-27-5
Crystal morphology	Acicular (needle-shaped)	Irregular	Orthorhombic
Purity	99.9%	99.9%	99.9%
Solubility in water (20°C)	1.40 g/L	1.33 g/L	1.10 g/L
Malvern particle size (Vol Mean Diameter)	39 µm	262 µm	185 µm

As seen in the above table, the sublimed, undensified molybdenum trioxide (Form A) was the product that was provided to NTP for testing. However, there are two very important considerations that need to be noted regarding this product and the question of potential exposure of California workers or the public. First, as noted above, this product is not generally sold commercially in any significant quantities, and information from our member companies shows that none of this product is sold into California. Secondly, and more importantly, the NTP did not test this “Form A” product directly. Prior to exposing the rats and mice, the undensified molybdenum trioxide was micronized in a Trost air-impact mill to average particle sizes ranging from 1.3µm for the 10 mg/m³ study to 1.5 µm for the 100 mg/m³ mice exposure study, and for the rat study, average particle sizes ranging from 1.5 µm for the 10 mg/m³ test series to 1.7 µm for the 100 mg/m³ study. This significant reduction in particle size performed by NTP resulted in essentially all of the molybdenum trioxide being available to the lower lung area, which is over 600 times greater exposure to the lower lung than if the actual, undensified molybdenum trioxide “Form A” itself had been used in the bioassay. The end result is that the product tested by NTP is not, nor will ever be, shipped into California or contained in industrial products used in the State.

Chemical Analysis of Molybdenum Compounds.

Any attempts to measure actual molybdenum trioxide exposure concentrations in the environment, workplace, soil or foods (if present at all) will result in the measurement of only the molybdenum element, thus making exposure and speciation determinations of the

molybdenum trioxide molecule nearly impossible under Proposition 65. The element molybdenum (Mo) can be analyzed by several methods, including Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Neutron Activation Analysis and Atomic Absorption and Emission Spectroscopy (USGS website). However, these methods simultaneously analyze at least 10 - 40 other metals and metalloids, thus making exact chemical speciation of various molybdenum compounds an impossible analytical challenge. Consequently, all molybdenum compounds, including molybdenum trioxide and even the forms that occur naturally in foods, can only be reported analytically as elemental molybdenum concentrations.

APPENDIX II: Background on Statistical and Biological Significance of NTP Cancer Bioassay Findings.

Dr. Joseph K. Haseman was the NIEHS/NTP Chief of the Biostatistics Branch and Director of Statistical Consulting during his 33-year career there (retired in 2004), and he was primarily responsible for the experimental design and data analysis of the NTP rodent carcinogenicity program. Many of his papers concerned the statistical design and interpretation of NTP cancer bioassay results, which provide an understanding of how NTP uses statistically significant findings in interpreting its cancer bioassays.

Two early papers by Haseman (1983, 1984) formed the statistical basis for the currently conducted NTP bioassay program. Haseman pointed out that NTP believes that no rigid statistical decision rule should be the sole basis for the ultimate decision regarding a chemical's carcinogenicity. From his review of long-term bioassay studies completed at that time, Haseman (1983) described a statistical decision rule that closely approximates the scientific judgment process used to evaluate these studies:

“Declare a compound carcinogenic if any common tumor showed a significant ($P < 0.01$) high-dose effect or if a $P < 0.05$ high-dose effect occurred for an uncommon tumor.”

Said another way, Haseman's (1984) decision rule was described as follows:

“...regard as carcinogenic any chemical that produces a high-dose increase in a common tumor that is statistically significant at the 0.01 level or a high-dose increase in an uncommon tumor that is statistically significant at the 0.05 level.”

In essence, the key points made by Haseman were to be aware that it is not appropriate to blindly regard every $P < 0.05$ statistically positive finding as a biological positive, and that when a common tumor is being evaluated, it must reach the $P < 0.01$ level if it is to have any potential biological relevance. Haseman urged that other non-statistical factors must be considered before a final judgment is made regarding the carcinogenicity of a chemical. This statistical thinking is what is still in place in the interpretation of the modern NTP bioassay as well, i.e., the final interpretation of the data should be based on biological judgment rather than on the rigid application of statistical decision rules.

Haseman and Elwell (1996) published a major paper on the evaluation of false positive (i.e., tumor increases due to random variability that are incorrectly judged to be chemically-related) and false negative (i.e., real chemically-related effects dismissed as random variability) bioassay results. In fact, this paper was published shortly before NTP completed the Technical Report on molybdenum trioxide. The authors stated that it was well recognized that a decision procedure that routinely considers all statistically significant tumor increases to be biologically meaningful will have an unacceptably high false positive rate. In addition, they noted that because of the large number of potential target sites evaluated in a typical rodent cancer bioassay, statistically significant ($P < 0.05$) chemically-related tumor increases may arise by chance. They also listed in their publication several reasons why the NTP has historically discounted some statistically significant tumor increases seen in the rodent bioassays. The most common reasons included:

“(1) the tumor increase was not dose-related (e.g., a significant increase was observed at a low dose but was not supported by an increase at other dose levels),

(2) the tumor increase, while statistically significant, was only marginally so and involved a high spontaneous incidence tumor,

(3) the concurrent control tumor response was abnormally low, and/or

(4) the elevated tumor response in the dosed group fell within the range of values considered normal for controls of that sex and species.”

The authors also defined in this paper the distinction between “common” tumors and “uncommon” (or rare) tumors occurring in rodents. “Common” tumors were defined as those tumor sites historically demonstrating a spontaneous rate greater than 2.0%, while the “uncommon” tumors occur at less than a 2.0% rate. They concluded that their analysis reflected “...the reality that common tumors are more likely to produce false positive outcomes than are uncommon tumors.”

In the evaluation of laboratory animal carcinogenicity studies, Haseman (1995) had earlier pointed out that while the statistical significance of an observed tumor increase is important, “...the final interpretation of rodent carcinogenicity studies should not be based on rigid statistical decision rules, but rather on the exercise of informed scientific judgment.” Additional factors cited by Haseman (1995) that should be used in judging the biological relevance of the findings included:

“(1) whether the effect was dose-related,

(2) whether the tumor increase was supported by an increase in related preneoplastic lesions,

(3) whether the effect was observed in other sex-species groups,

(4) whether the effect occurred in a suspected target organ, and

(5) the historical control rate of the tumor in question.”

History of Chemically Induced Lung Lesions in NTP Bioassays.

NTP researchers recently published a comprehensive review and evaluation of all the lung tumor findings in 545 peer-reviewed NTP studies published to date (Dixon et al., 2008). They reported that the lung is the second most common target site (liver is the first) of neoplasia of the chemicals tested, with 64 chemicals in 66 reports producing significant neoplasias in the lungs of rats and/or mice (defined as “clear,” “positive” or “some” evidence). Molybdenum trioxide was included in their analysis. Of the studies associated with lung tumor induction, approximately 35% were inhalation and 35% were gavage studies, with dosed-feed, dosed-water, topical, intraperitoneal or *in utero* routes of administration accounting for 18%, 6%, 3%, 1%, and 1% of the studies, respectively. The most commonly induced lung tumors were alveolar/bronchiolar (A/B) adenoma and/or carcinoma for both species, while the most frequently observed nonneoplastic lesions included hyperplasia of the

alveolar epithelium and inflammation in both species. The liver was the most common primary site of origin of metastatic lesions to the lungs of mice.