Commentaar op conceptadvies molybdeen en anorganische molybdeenverbindingen

Comments on draft report Molybdenum and selected inorganic molybdenum compounds

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Dit document bevat de letterlijke weergave van de commentaren (deels Engelstalig) van:

- IMOA, *International Molybdenum Association*
- NIOSH, *National Institute for Occupational Safety and Health*

This document contains the comments (one in Dutch) by:

- IMOA, *International Molybdenum Association*
- NIOSH, *National Institute for Occupational Safety and Health*

THE VOICE OF THE MOLYBDENUM INDUSTRY

IMOA response to: Draft advisory report for public review: Molybdenum and selected inorganic molybdenum compounds Health Council of the Netherlands, 2 September 2024

For the attention of:
Dr. R.H. Mennen
Subcommittee on the Classification of Substances Toxic to Reproduction The Health Council of The Netherlands
(draftOSH@gr.nl)

27 November 2024

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Kindly respond to the
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make this submission

THE VOICE OF THE MOLYBDENUM INDUSTRY

Dear Dr. Mennen,

The International Molybdenum Association (IMOA) has reviewed the DECOS Draft Advisory Report for public review, dated 2 September 2024, entitled *"Molybdenum and selected molybdenum compounds – Evaluation of the effects on reproduction, recommendation for classification".* For the reasons summarized here below and the substantial weight of evidence explained more fully in the attached documents, IMOA strenuously disagrees with the DECOS evaluation of Category 2, suspected human reproductive toxicant [H361f].

IMOA's molybdenum industry membership takes the matter of any possible health concerns from molybdenum products extremely seriously, and has therefore further investigated reported findings of potential reproductive toxicity from earlier preliminary research, amongst others Pandey & Singh 2002[1]. IMOA's investigations are conducted according to EU REACH-mandated OECD internationally recognised test protocols, e.g. OECD 416 Two-Generation Reproduction Toxicity study, to generate statistically and scientifically robust data.

There are three integral components to our response to the DECOS Draft Advisory Report: 1) this cover note, 2) the Expert Review by Dr. Creasy, and 3) the tabulated list of further detailed comments on the DECOS draft report.

As an inherently necessary part of this process, IMOA commissioned Dr. Dianne Creasy, a highly experienced and internationally renowned toxicologic pathology consultant and expert in male reproductive assessments to undertake a comprehensive review of the reproductive toxicology studies and published literature which form the basis of the DECOS recommendation to classify molybdenum and selected inorganic molybdenum compounds as suspected to be a reproductive toxicant to humans (H361f, suspected of damaging fertility).

Dr. Creasy has reached the conclusion that the several toxicity studies performed according to OECD/NTP guidelines in GLP compliant laboratories (NTP 1997, Murray 2014, 2019) very clearly stand out as providing the most reliable and statistically robust weight-of-evidence data on which to base any hazard classification assessment of molybdenum compounds. Based on her expert critical assessment of the various other investigative studies of molybdenum toxicity cited by DECOS, Dr. Creasy has set out in her Expert Review why those are unreliable and the conclusions are unsupported.

Consequent to this expert view and extensive analyses of the data, IMOA has therefore reached a different substantiated conclusion, that of 'no hazard classification required', in sharp contrast to the 2024 DECOS draft proposal to classify for adverse effects on sexual function and fertility based on unreliable, inadequate data for regulatory purposes. This is because:

a) The reported findings in the Pandey & Singh and Jeter & Davis[2] publications which largely formed the basis of the earlier DECOS 2013 classification proposal (Category 2, suspected human reproductive toxicant [H361f]), have not been

[1] Pandey R and Singh S. *Effects of molybdenum on fertility of male rats*. Biometals 2002; 31 15: 65-72.
[2] Jeter MA and Davis GK. *The effect of dietary molybdenum upon growth, hemoglobin,* 9 *reproduction and lactation of rats*. The Journal of Nutrition 1954; 54(2): 215-20.

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replicated in more recent high quality GLP compliant, OECD Guideline studies using similar *and higher* dose levels.

b) Furthermore, the attached Expert Review reveals that key histopathological reported findings in Pandey & Singh are in reality not evidenced in the study.

It is likewise highly noteworthy and relevant that the scientific opinion of The European Food Safety Authority also concluded upon the need to disregard for regulatory purposes a similar unreliable study by Pandey & Singh where nickel was the focus substance. Specifically, the EFSA conclusion was: *"Limitations in these studies preclude their use for the establishment of a reference point."*[3]

c) The DECOS draft proposal states: 'It should be noted that there are limitations in reporting of some of the studies'. For IMOA, and based on the available peer-reviewed published science now in 2024, that is a major understatement and gross over-simplification. There are numerous serious inadequacies with the other studies (i.e. those not GLP OECD test protocol-compliant) which cannot be characterized as mere 'limitations in reporting', e.g. inadequate experimental design (particularly the numbers of animals investigated), unspecified or uncertain methodology, failure to report the test material and dose levels, inadequate measures of systemic toxicity, incomplete, inadequate and inaccurate reporting of data, etc. All the above are pinpointed by Dr. Creasy in her attached *Expert Review of the Technical Validity of the basis proposed by the Health Council of the Netherlands to classify molybdenum and selected inorganic compounds as suspected reproductive toxicants to humans,* and in the tabulated comments.

d) In its report DECOS states on page 8: 'The dose levels used in the studies were considered to be low and the results at similar dose levels were contradictory'. IMOA finds this an overly simplistic and partial evaluation statement, which is mis-leading because the dose ranges used (see table overleaf) in the OECD test protocol-compliant studies consistently demonstrated an absence of reproductive toxicity. Moreover, the latter used dose ranges of this biological essential element not just similar to, but importantly also far exceeding, those of the non-GLP, non-OECD test protocol-compliant studies.

e) IMOA notes the dose levels reported by DECOS are not consistently expressed as mg Mo/kg/day across the various molybdenum salts investigated, which might go some way to explain why DECOS states 'similar dose levels'. Dose ranges are much wider and higher in the GLP,OECD/NTP test protocol-compliant studies that also benefit from optimal designs, more statistical power (larger numbers/groups of animals), higher dose levels, more sensitive and reliable measurements of male reproductive toxicity by accepted and validated methods, and better-informed interpretation of test results.

[3] EFSA Panel on Contaminants in the Food Chain (CONTAM), 2020. Update of the risk assessment of nickel in food and drinking water. EFS2 18. https://doi.org/10.2903/j.efsa.2020.6268

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IMOA therefore strenuously asserts that now in 2024 the currently available weight of evidence does not justify a Category 2 H361f proposal by DECOS, not least because the additional high-quality data of the 2019 peer-reviewed published 2-generation reproductive toxicity study[4] with two high dose levels by different routes and hence far superior statistical power has not identified any adverse effects and not reproduced the results of the earlier inadequate, technically deficient and unreliable studies.

IMOA agrees with DECOS that "systemic toxicity correlates with the ability of the substance to release molybdate ions" and notes that there are several poorly soluble / insoluble molybdenum substances used at workplaces, such as the metal itself (on its own or in alloys), and molybdenum disulfide. Therefore, any hazard classification assessment should make a clear distinction between highly soluble molybdenum salts being in scope, with poorly soluble/insoluble substances being out of scope.

We also clarify that neither ammonium tetrathiomolybdate, nor other tetrathiomolybdates (TTM) should be included in the "molybdate" read-across/category as the chemistry and biology of thiomolybdates is different from that of oxomolybdates. TTM is a potent copper chelator and a molybdenum-sulfur compound that behaves differently from molybdenum-oxygen compounds, and is therefore not representative of the soluble molybdate-releasing compounds that form the category.

On pages 5 & 6 of this document IMOA has provided dose levels adjusted for the molybdenum content of the specific salt administered, to facilitate appropriately accurate comparison of outcome by molybdenum dose administered. For example, sodium molybdate (dihydrate) contains 40% molybdenum by weight, diammonium dimolybdate 56% and molybdenum trioxide 67%. For those publications where the identity of the test material is not clearly identified, e.g. described only as 'molybdenum' when it is likely that a soluble salt has been administered, it should be noted that dose levels may be overstated.

As our closing remark therefore, we respectfully submit that it is unwarranted that unsupported conclusions of unreliable studies are used by DECOS as a preferred basis to seek hazard classification, when recent high-quality peer-reviewed published OECD protocol guideline-compliant GLP studies with higher statistical power at higher dose levels are available that must be appropriately taken into account, and which substantiate a conclusion of 'no hazard classification required'. The EU REACH Regulation legally requires testing according to stringent OECD and other internationally recognised protocols and IMOA has complied with that legal requirement. For DECOS to have drafted their 2024 classification proposal and evidently not given the quality, guideline-compliant studies the appropriate weight-of-evidence they merit is to undermine and reduce that legal requirement to an absurdity.

[4] Murray FJ, Sullivan FM, Hubbard SA, Hoberman AM and Carey S. *A two-generation* 22 *reproductive toxicity study of sodium molybdate dihydrate administered in drinking* 23 *water or diet to Sprague-Dawley rats*. Reproductive Toxicology 2019; 84: 75-92.

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IMOA therefore thanks you in advance for your careful scrutiny of our three-part response prior to re-visiting your current draft, and please be assured we are available for further dialogue on this important issue.

Yours sincerely
Sandra Carey
IMOA HSE Executive

Table of key studies (full references in DECOS draft advisory report, pages 78-89), expressing all dose levels in mg Mo/kg bw/day:

Study author/year	Dose ranges and conversion to mg Mo/kg bw/day:	Summary in mg Mo/kg bw/day
OECD test protocol-compliant GLP studies:		
Murray 2019 (2-generation study):	Drinking Water: **0, 5, 7, 40 mg Mo/kg bw/day** Diet: **0, 40** mg Mo/kg bw/day Both routes administered as SMD	Drinking water: **0, 5, 7, 40** Diet: **0, 40**
Dose range-finder for Murray 2019:	Diet**: 0, 3, 20, 40 mg Mo/kg bw/day** Drinking Water**: 0, 3, 20, 40** mg Mo/kg bw/day. Both routes administered as SMD	Diet: **0, 3, 20, 40** Drinking Water: **0, 3,20,40**
Murray 2023:	Drinking water: **0, 20, or 40 mg Mo/kg bw/day (**as SMD) at 0, 169–235 or 337–432 ppm	Drinking Water: **0, 20, or 40**
Murray 2014 (90-day repeated dose):	Gavage: **0, 5, 17, 60 mg Mo/kg bw/day** (as SMD)	Gavage: **0, 5, 17, 60**
Range-finder for Murray 2014	Gavage: **0, 4, 20, 2x10 mg Mo/kg bw/day** Diet: **0, 4, 20 mg Mo/kg bw/day** Both routes administered as SMD	Gavage: **0, 4, 20, 2x10** Diet: **0, 4, 20**
NTP 2-year toxicology study, 1997:	In the 104 week Rat NTP study blood levels of Mo were: 0.22, 0.80, 1.77 and 6.04 µg/g for 3, 10, 30 and 100 mg $MoO^3/m^{3,}$ respectively, for males. Exposure levels (as blood levels) for males at the high dose in this inhalation study can therefore be compared to week 12 of the 90-day study where they are higher than the 2930 ng/mL (2.93 µg/g) reported for 17 mg Mo/kg bw/day and slightly lower than the 9903 ng/mL (9.9 µg/g) reported for 60 mg Mo/kg bw/day.	Inhalation: **>17, <60**
Non OECD test protocol-compliant non GLP studies:		
Jeter & Davis, 1954:	Diet: 0, 5, 20, 80, 140 ppm. Equivalent to 0, 1.8, 7.2 and 12.6 mg SMD/kg bw/day or **0, 0.72, 2.88 or 5.04 mg Mo/kg bw/day** [subchronic rat conversion factor 0.09, EFSA 2012].	Diet: **0, 0.72, 2.88 or 5.04**
Schroeder, 1971:	Drinking Water: 10 ppm 'molybdate' for mice is equivalent to **1.5 mg/kg bw/day f**or subchronic exposure [EFSA 2012]. If the test material was SMD, the level of molybdenum would be even lower at 0.6 mg/kg bw/day.	Drinking Water: **0, 1.5 or 0.6**
Fungwe, 1990:	Drinking Water: 0, 5, 10, 50, 100 mg/L SMD, equivalent to 0.09, 0.45, 0.9, 4.5 and 9.0 mg SMD/kg bw/day or 0, **0.18, 0.36,1.8 and 3.6 mg Mo/kg bw/day** [rat subchronic conversion factor	Drinking Water: **0, 0.18, 0.36,1.8 and 3.6** (actual substance administered unclear)

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	0.09, EFSA 2012]	
Howell, 1993:	Drinking Water: 130.29 µmol Mo/litre as ammonium molybdate, 260.58 µmol Mo/litre as tetrathiomolybdate [No body weight or water intake data reported and no conversion factors available. Ammonium molybdate estimated **as 5 mg Mo/kg bw/day based** on drinking water consumption by guinea pig of 200 mL/kg/day. 261 µmol/L (*96 µg/µmol) --> 25000 µg/L (*0.2 L/kg/day) = 5 mg/kg/day]	Drinking Water: **0**, **5**
Pandey & Singh, 2002:	Gavage: 0, 10, 30, 50 mg SMD/kg bw/day; **0, 4, 12, 20 mg Mo/kg bw/day** [reported by ATSDR as 0, 4.7, 14, 24 mg Mo/kg bw/day]	Gavage: **0, 4, 12, 20**
Zhai, 2013:	Drinking Water: 0, 12.5, 25, 50, 100, 200 mg SMD/L is equivalent to 0, 2.25, 4.5, 9.0, 18.0, 36 mg SMD/kg bw/d or 0, **0.9, 1.8, 3.6, 7.2, 14.4 mg molybdenum/kg bw/day** [conversion factor 0.18 for mouse subacute, EFSA 2012]	Drinking Water: **0, 0.9, 1.8, 3.6, 7.2, 14.4**
Zhang, 2013:	Drinking Water: 0, 5, 10, 20, 40 mg SMD/L is equivalent to 0, 0.9, 1.8,3.6,7.2 mg SMD/kg bw/d or 0, **0.36, 0.72, 1.44, 2.88 mg Mo/kg bw/day** [conversion factor 0.18 for mouse subacute, EFSA 2012]	Drinking Water: **0, 0.36, 0.72, 1.44, 2.88**
Wang, 2016:	Drinking Water: 400 mg/L, equivalent to **60 mg/kg bw/day of 'molybdenum'** – not further described [mouse subchronic conversion factor of 0.15, EFSA 2012]	Drinking Water: **0, 60** (actual substance administered unclear)
Khorami, 2020:	Gavage: 0, 0.05, 0.1, 0.2, 0.4 mg SMD /kg bw/day, **0, 0.02, 0.04, 0.08, 0.16 mg Mo/kg bw/day**	Gavage: **0, 0.02, 0.04, 0.08, 0.16**

Note: Mo =molybdenum, SMD = Sodium Molybdate Dihydrate Conversion based on 40% Mo in SMD by molecular weight.

Enc.
Expert Review by Dr. Dianne Creasy
IMOA Tabulated Response to DECOS (draft advisory report)

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Expert Review of the technical validity of the basis proposed by the Health Council of the Netherlands to classify molybdenum and selected inorganic molybdenum compounds as suspected reproductive toxicants to humans

Introduction

This document provides a review of selected literature pertaining to the male reproductive toxicity of molybdenum with particular emphasis on studies that provide information on anatomic toxicologic pathology and sperm analysis carried out in rodents. Studies available in published literature provide conflicting data on the potential for molybdenum compounds to produce male reproductive toxicity when administered to rodents for different periods of time. The quality of the studies varies markedly in the reliability of the methodology and data, as well as the types and doses of molybdenum compounds tested and the endpoints measured to investigate reproductive toxicity.

Identification and evaluation of male reproductive toxicity is complex and requires integration of information from a variety of endpoints. Male infertility can be mediated through numerous different mechanisms, and so an integrated assessment of as many endpoints as possible (organ weights, histomorphology, sperm parameters, hormonal measurements, and fertility endpoints) provides the most thorough evaluation. In most cases, multiple endpoints will be affected. Morphological assessment of spermatogenesis requires particular expertise and needs to be carried out on well-fixed tissue (Modified Davidson's or Bouins) and with an in-depth knowledge of the spermatogenic cycle, otherwise fixation artifacts and normal features of tubules in different stages of the cycle can be mistaken for degenerative changes.

The first part of the review will discuss the important aspects of male reproductive toxicity investigations and address the difficulties and pitfalls of interpretation, using individual research papers contained in this molybdenum toxicity review to illustrate where appropriate methodology and experience have been employed or where mistakes or poor study design have led to unreliable or incorrect interpretation. The second part of the review will briefly assess the strengths and shortfalls of the individual studies on molybdate toxicity that I have reviewed (see Appendix 1). My conclusions are provided in the final section.

Extent of This Review

Numerous toxicity studies and research investigations have been published on the effects or absence of effects of molybdenum compounds on male reproductive parameters. The studies evaluated in this review are listed in Appendix 1. Some of the published studies were performed to strict regulatory guidelines (OECD, FDA or NTP), which dictate: 1) that group sizes are adequate to provide statistically robust analysis, 2) that methodology for performing procedures such as sperm analysis, histopathological fixation/evaluation and fertility assessment are performed to "best practice" standards, and 3) that reporting of data is comprehensive and complete. Such studies include NTP 1997 and Murray 2014, 2019. These studies were also performed under GLP regulations, which require that any methodology used has been validated for the species being tested and the personnel involved in the study are suitably qualified and experienced. Other published studies on molybdate toxicity were often small research projects using inadequate numbers of animals, poorly detailed and basic methodology, inappropriate

fixatives for testicular histopathology and incomplete or inadequate reporting of data (for example, Jeter 1954, Pandey 2002, Zhai 2013, Wang 2016).

Molybdenum exists in many forms and can be administered via different routes (oral gavage dosing, in the feed, in the water, inhalation, intraperitoneal (IP) injection). The studies included in this review used different compounds of molybdenum with varying routes of dosing, different dose levels and different durations of dosing, making it difficult to compare results across studies. In addition, to investigate molybdenum interaction with copper, some studies were performed with copper depleted and copper supplemented diets to investigate the role of copper in molybdate toxicity (Jeter 1954, Wang 2016). These variables added to the complexities of comparing results between studies. Where possible, dose levels reported for each salt have also been expressed in mg Mo/kg bw/day to enable some comparison of exposure between studies.

Part 1: Important Considerations for Toxicologic Examination of the Male Reproductive System

The main endpoints available for detecting toxicity in the male reproductive system of laboratory species are organ weights, histopathology (considered the most sensitive), sperm analysis, and fertility (considered the least sensitive) (Creasy 2013, Vidal 2014). For studies investigating potential modes of action (MOA) of toxicity, hormone measurements and oxidative stress markers can also be useful (Creasy 2013).

In general, the more endpoints that are measured, the more data there is available for integration and evaluation. For example, studies such as Schroeder 1971, where fertility was assessed only by the outcome of mating and survival of offspring over several generations, but where clinical condition was not assessed and decedents were not examined cannot discriminate between direct effects of the chemical on reproductive function versus indirect effects caused by loss of body weight, poor clinical condition and low food intake. Reproductive Assessment by Continuous Breeding (RACB) , which was successfully used by the National Toxicology Program for many years (Chapin 1997), also utilised a study design with continuous breeding over several generations but in addition to fertility outcome and litter size/weight, it incorporated many additional endpoints for evaluating male and female reproductive toxicity in a stepwise manner. These including body weights, food intake, organ weights, sperm parameters and oestrous cyclicity, as well as histopathology.

Organ weights

Seminal vesicle and prostate organ weights are very useful and sensitive endpoints for detecting hormonal disturbance. These are androgen dependant organs that rely on testosterone for regulation of secretory activity. Even small decreases in circulating testosterone will generally be reflected by a decrease in the weight of prostate and seminal vesicle. In fact organ weight is a more sensitive indicator of low androgen than morphological examination of these tissues.

Testis weight is generally much less sensitive than morphological examination as an endpoint for toxicity, but an important requirement when evaluating testis weight is that absolute weight should be used rather than testis weight relative to body weight. Similar to the brain, testes maintain their weight despite body weight loss (Creasy 2003, 2013), so drawing conclusions regarding changes in relative testis weights in preference to absolute testis weights (as reported in Pandey 2002) is inappropriate.

Epididymal weight is also a useful endpoint, which reflects sperm content as well as androgen status. Low testosterone will generally decrease epididymal weight as will reduced sperm content. Sperm content is responsible for approximately 30% of the weight of the epididymis, so a significant decrease in epididymal weight should generally be accompanied by a decrease in sperm content (by histopathology) or sperm count (by sperm assessment), and vice versa. This underlines the importance of integrating multiple endpoints when evaluating potential toxicity.

The organ weights of the accessory sex organs (prostate and seminal vesicles) including their secretions are particularly useful for assessing the androgen status of the animal. The secretory function of these tissues is androgen dependant and any decrease in testosterone levels or metabolism of testosterone to dihydrotestosterone will be reflected by a decrease in organ weight. Organ weight of these tissues is a sensitive endpoint for androgen status and is more sensitive than histopathology.

In any case of male reproductive assessment, it is important to present body weight and organ weight data so that it can be correlated with other endpoints of toxicity to help support or dismiss equivocal results.

Histopathological evaluation of testes

Histopathology is considered the most sensitive endpoint for detecting testicular toxicity (Ulbrich 1995, Vidal 2014), but only if the testes are fixed correctly and the testes are evaluated in a stage-aware manner (Creasy 1997, Lanning 2002, Latendresse 2002, Creasy 2003). Compared with most other tissues, the testis is a fragile tissue and sensitive to poor handling and processing artifacts during preparation for histopathology sectioning. All regulatory guidelines relating to the detection of testicular toxicity recommend fixation (whole) in either Bouin's fixative or Modified Davidson's fixative (formalin should not be used). Even when the correct fixative is used, poor handling and processing can result in significant artifacts and deterioration of the delicate tubular structures and the orderly germ cell organization. This can be seen in the photomicrographs of testes presented by Pandey 2002 and Wang 2016 , which were fixed in formalin or paraformaldehyde, where the structure of the seminiferous tubules is so distorted and fragmented that evaluation of any abnormal changes would be extremely difficult if not impossible. A number of the investigative studies also took multiple samples from the testis, cutting it into pieces before fixing it, this destroys the normal orderly architecture of the seminiferous tubules and causes damage and sloughing of germ cells, which can easily be mistaken as induced damage. Best Practice guidelines all recommend fixing the testis whole (Lanning 2002, Kittel 2004).

When evaluating the testes, it is necessary for the pathologist to have a good understanding of the normal artifacts that accompany different fixatives (Latendresse 2002). For example, Bouin's and modified Davidson's are both good fixatives for the germinal epithelium, but cause significant shrinkage of the tubules, often resulting in fluid accumulation in the intertubular space. This is a normal fixation artifact, but occurs differentially, with more shrinkage and fluid occurring towards the centre of the testis and less towards the periphery. This can easily be mistaken for test article-related shrinkage. However, Khorami 2020 fixed testes in Bouins and the photomicrographs in the paper show similar tubular shrinkage due to fixation artifact, but (correctly) do not consider this abnormal.

The experienced toxicologic pathologist will also be familiar with the multitude of other artifacts that are routinely seen in histopathologic sections including those caused by rough handling

during necropsy (causing exfoliation/sloughing of germ cells into the tubular lumen) or poor processing (causing patchy staining and apparent "loss" of germ cells. These variable and sporadic artifacts can be easily mistaken as abnormal histopathologic findings, which is why it is important for the investigator to be experienced in evaluation of the tissue, but more importantly to record and tabulate the incidence, severity and nature of any treatment-related findings that they consider to be present. None of the studies claiming to show histopathologic findings in the testis or epididymis, present this data.

Another important feature of the testis that needs to be considered by the pathologist when evaluating seminiferous tubules, is the stage of spermatogenesis (Creasy 1997, 2003, Lanning 2002). Spermatogenesis progresses in the seminiferous tubules in a precise and highly ordered pattern with 4 generations of germ cells developing in synchrony with one another. When examining a complete cross section of rat testis, each profile of seminiferous tubule (of which there would be approximately 500 in one section) will be in a particular stage of the spermatogenic cycle, which is defined by the precise cell types present in that tubule. Fourteen different stages of spermatogenesis are recognized in the rat testis and these 14 stages are all different in terms of their appearance and the cell types that are present. Some tubules will have prominent sperm at the surface, waiting to be released, while others will look totally different and have no sperm at the lumen, because they have recently been shed. Unless the pathologist understands the spermatogenic cycle and can recognize the different stages of spermatogenesis, they can wrongly compare different stage tubules and conclude that abnormalities are present when they are not. For example, tubules in some stages (stages V-VIII) will contain prominent bundles of sperm at the lumen. The sperm is released into the epididymis during stage VIII, resulting in later stage tubules being devoid of sperm. In addition, some tubules (stage IX-XI) have prominent cellular debris in the form of residual bodies, which can easily be mistaken for degenerating germ cells by an inexperienced investigator. Studies performed to regulatory guidelines are evaluated by Board Certified Pathologists who have been trained in veterinary pathology and understand the complexities of histopathological evaluation.

Normal Background Pathology

All organs and tissues, regardless of species have a selection of histopathological abnormalities that are part of the background profile of that tissue and that species. It is important that the toxicologic pathologist is aware of the types of changes seen in untreated controls and of their expected incidence.

The incidence of background pathology in rat testes is generally quite low but it will vary with the strain and age of the rat. Degenerative testicular lesions are more common in mice testes but again this depends on the strain. The incidence and severity of background lesions can be increased by a reproductive toxicant, but this can only be demonstrated if the incidence and severity of the findings are recorded so that any dose response and relationship to treatment can be evaluated. This is standard procedure in regulatory, OECD guideline-compliant toxicity studies, such as those reported by NTP 1997 and Murray 2014, 2019, but all of the other investigative studies reviewed in this report failed to provide the incidence or severity of any of the changes they reported in the reproductive tissues. Frequently, the only evidence provided was one or two photomicrographs purporting to show an abnormality.

Another aspect of "normal background pathology" that can cause misinterpretation, relates to sexual maturation status. Immature and prepubertal testes contain large numbers of

degenerating germ cells, sloughed germ cells and missing germ cells. Not all animals will attain sexual maturity at the same age and so there can be marked differences between the size and appearance of testes in a cohort of peripubertal animals in a study. Wang 2016 used mice that were of an age that would be sexually immature or peripubertal, and so measuring sperm parameters would be subject to variations depending on the individual maturation state of the mouse. Studies investigating testicular toxicity should always be carried out using sexually mature animals (Lanning 2002, Creasy 2003).

Sperm Analysis

The main parameters measured during sperm analysis are sperm count/concentration, sperm motility and sperm morphology. Changes in sperm count generally reflect changes in output from the testes, changes in motility can be due to disturbances in spermatogenesis or in epididymal function and changes in sperm morphology generally reflect abnormalities in spermatid maturation in the testis. The measurements are routinely made on sperm taken from the cauda (tail) of the epididymis or the vas deferens. An important consequence of the site of sampling is that the sperm measurements come from sperm that were released from the testis approximately 2 weeks earlier, because this is the average transit time taken for sperm to reach and be stored in the cauda. This fact has not been taken into consideration in the paper by Zhai 2013, where sperm analysis was performed on sperm sampled after 2 weeks of dosing. Therefore any changes in sperm parameters would be predicted to be due to effects on epididymal function, but the authors relate the sperm changes to alterations in testicular enzymes.

Wang 2016 sampled sperm from the whole epididymis rather than the cauda and vas deferens; this would result in the sample being contaminated with immotile and immature sperm from the body and proximal portion of the epididymis. Sperm count should be expressed as 10^6 per g of cauda tissue sampled (see NTP 1997, Murray 2014), but Pandey 2002, sampled sperm from a portion of the cauda and then expressed the count per epididymis, which doesn't take into account the variable weight of tissue sampled.

In rats, sperm count and motility show quite a lot of variability between animals whereas the number of sperm with abnormal morphology are normally very low. The degree of variability in sperm parameters means that adequate numbers of animals need to be used to achieve statistically robust data. A minimum of 10 animals/group should be used and preferably 20/group. Many of the investigative studies reviewed fell short of this. There are also strain differences, so when evaluating sperm parameters for test article related changes it is important to have a good historical control data base (HCD) as a reference point for questionable results. This is particularly important when using small group sizes so that small changes, that are within normal control variation, are not over-interpreted. Most sperm analysis is performed using CASA automation, except for sperm morphology which is still a manual technique. CASA is well validated and provides standardized results compared with manual methods using hemocytometers, which rely on operator skill/experience. The results reported by Murray 2014, illustrate the importance of historical control data (HCD) for evaluating a small apparent reduction (59.0% vs 69.4% in controls) in progressive sperm motility that occurred in the 60 mg Mo/kg bw/d molybdenum-treated animals at the end of dosing. Reference to the HCD maintained by the conducting laboratory demonstrated that the progressive motility for the control animals (69.4%) was at the upper end of the recent historical control values while the progressive motility measurements of the treated animals, although lower (59.0%), were well within the HCD and close to the historical control mean (59.8 ± 16.2%). This explanation for the

difference in this one parameter is the most likely since there was no corresponding change in total sperm motility in this study or in either generation of the 2 generation study (Murray 2019).

Oxidative Stress Markers/ Testicular Enzymes

A number of studies reviewed, used testicular enzymes such as lactate dehydrogenase, acid and alkaline phosphatase and sorbitol dehydrogenase, or markers of oxidative stress including malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx) superoxide dismutase (SOD) etc) as indicators of testicular toxicity (Pandey 2002, Zhai 2013, Wang 2016, Khorami 2020). These are not routine endpoints for detecting testicular toxicity, and are not recommended in any regulatory guidelines. While they can be useful adjuncts for investigating possible mechanisms of toxicity, they are inadequate when used on their own, because it is essential that any changes in enzyme levels can be shown to correlate with morphological or functional injury. Although these enzymes may show treatment-related changes, in response to molybdenum exposure, the changes may only reflect a physiological adaptive response to the presence of higher than normal levels of the essential nutrient molybdate. The change is only toxicologically important if it is accompanied by good evidence of morphological or functional injury, indicating that the protective enzyme responses have been exceeded. In most of the research studies where oxidative enzymes were measured, adequate evidence of morphological or functional injury was lacking. So changes in testicular enzymes on their own do not indicate physiologically relevant injury.

While most of the studies that measured oxidative stress enzymes reported a molybdenum-related decrease in the protective enzymes and an increase in lipid peroxidation products, Khorami 2020 reported no changes in these enzymes when molybdenum was administered on its own, but increased levels of protective enzymes and decreased MDA levels when molybdenum was given to rats in association with the testicular toxicant cadmium chloride ($CdCl_2$).

Another shortcoming of the studies reporting changes in oxidative stress enzymes is the lack of any mention of validation for the assay methodology or of the kit used. As with hormone measurement (discussed below), validation of methodology is an important prerequisite for such measurements. As discussed by Griffiths 2002 and Collins 2005, there are numerous problems associated with choosing the correct assay to measure oxidative stress markers and making sure that the assay is validated and sensitive in the species being tested. In a comprehensive review of the subject, Griffiths (2002) states *"One of the key issues faced in the analysis of any oxidation product is the validity of the assay. The following criteria should be met: (i) identity, (ii) linearity, (iii) accuracy, (iii) selectivity, (iv) reproducibility and (v) an appropriate limit of detection. In addition, normal ranges should be established for each assay, reflecting age and sex of the subject group."* Validation is a pre-requisite of any assay before it can be used in a GLP-compliant laboratory for a GLP compliant study. It should also be an essential requirement in any laboratory that expects to obtain reliable and trustworthy results from any Biomarker Assays. However, there was no evidence that such assay validation was performed in any of the studies that reported measuring oxidative enzymes.

Hormone Analysis

In situations where male reproductive toxicity has been demonstrated, studies are sometimes performed to investigate the role of hormones in the MOA. However, because of the pulsatile nature of reproductive hormone secretion and their notorious variability in rodents, large group

sizes need to be used to overcome the inherent coefficient of variation between measurements. For example, Pierroz 1999 demonstrated that the testosterone values in an untreated individual rat can vary between <1 and 16 ng/mL over a 5 hr period. Based on this variability, it has been calculated that a group size of ≥20 is needed to stand a reasonable (80%) chance of detecting a moderate (25%) change in testosterone levels (Chapin 2012). Investigative studies that set out to measure reproductive hormones often use group sizes of <10, but such studies are severely underpowered to detect the hormonal changes they claim; in fact such small group sizes would stand less than a 17% chance of detecting even a 25% change in testosterone (Chapin 2012) and so such results are misleading and completely unreliable. Another common shortcoming of studies performing hormone analysis is the failure of the investigators to validate the methodology and the kit used for analysis. Frequently, the kit used to measure rodent hormones by immunoassay has been developed to measure human hormones and there may be limited or no cross-reactivity with rodent hormones. Validation of the assay kit and the methodology is an essential step in producing reliable results for such assessments.

Effects of stress and reduced body weight/food intake on reproductive parameters

In general, stress and reduced food intake have few effects on spermatogenesis and testicular histopathology, but androgen secretion is sensitive, and will be reduced by relatively mild decreases in food intake (O'Conner 2000, Rehm 2008, Everds 2013). Although the decreased testosterone resulting from low food intake has little qualitative effect on spermatogenesis, it will cause reductions in germ cell numbers (specifically pachytene spermatocytes and round spermatids in stage VII/VIII tubules) (see Creasy 2001, Rehm 2008). In addition a reduction in weight of the seminal vesicles and prostate is a sensitive indicators of reduced testosterone and sperm production and epididymal weight are also often decreased. The effects on testosterone are more marked in mice than in rats (see Everds 2013 for review). Based on this, it is important to have data relating to the food intake, body weight and clinical condition of animals exposed to a substance, when evaluating reproductive parameters for toxicity so that this can be taken into account as a confounding factor. This was a surprising omission from a number of the papers including Pandey 2002, Zhai 2013, Wang 2016, Khorami 2020.

Chemical formulation, route of administration and duration of exposure of administered molybdenum

Various formulations of molybdenum have been used in the toxicity studies evaluating the effects of the mineral on reproductive parameters. Sodium molybdate (39% molybdenum) and ammonium molybdate (54% molybdenum) are the two most common salts used, but molybdenum trioxide (67% molybdenum) (NTP 1997) has also been studied. Wang 2016 did not specify the chemical form of molybdenum used, which they administered along with copper but failed to provide dose levels (in mg/kg bw/d) of either. The formulation and route of administration will have a significant impact on the rate and efficiency of molybdenum absorption and its distribution as well as on its potential toxic effects. For example, sodium molybdate was shown to increase serum levels of copper (Murray 2014, 2019) and increase its excretion from the body. In a number of the literature studies investigating molybdenum toxicity, copper levels were also modified, and often administered in non-standard diet, adding to the difficulties in comparing results to the GLP compliant studies where commercial standard rodent diets with verified copper levels were consumed.

In the studies reviewed, molybdenum was administered by various routes. In some it was incorporated in the drinking water (Zhai 2013, Wang 2016, Murray 2019) or in the diet (Jeter 1954, Murray 2014, 2019). In others it was administered by oral gavage (Pandey 2002, Khorami 2020) and in one study by inhalation (NTP 1997). The toxicokinetics (Cmax and AUC) of molybdenum from the different methods of dosing may vary dramatically and in order to make meaningful comparisons between studies it is necessary to know the serum concentration of molybdenum during the study. This was addressed in the studies by Murray 2014 who measured serum and blood levels of molybdenum during week 4 and week 12 of the 13 week dosing period as well as during the recovery period. Murray 2019 also measured serum and blood levels of molybdenum in the different generations of rats in the 2 generation reproductive study and NTP 1997 measured blood levels of molybdenum in the 104 week inhalation study with molybdenum trioxide. None of the other studies reviewed here measured systemic levels during the studies. Pandey 2002 measured testicular levels of molybdenum but this doesn't allow comparison of circulating levels of Mo in the different studies.

When administering molybdenum in the diet or drinking water, in order to ensure animals receive the intended and stated dose level, formulations of the diet and drinking water for the different dose levels must be prepared at regular intervals (defined by stability analyses) throughout the study based on the mean body weight and food or water intake of the animals in that group. This procedure was followed in the studies by Murray 2014, 2019 but other studies (e.g. Jeter 1954, Zhai 2013, Wang 2016) failed to measure/report body weight, food or water intake, making it difficult to establish Mo exposure levels.

The duration of molybdenum administration varied between studies, making it difficult to make meaningful comparisons. Of the studies reviewed here, dosing duration varied from 14 days (Zhai 2013) up to 100 days (Wang 2016). In general, the longer the duration of dosing, the more severe any toxicologically induced injury would be expected to be.

Part 2: Individual Study Reviews

Pandey, R., Singh, S.P., 2002. Effects of molybdenum on fertility of male rats. BioMetals 15, 65–72. https://doi.org/10.1023/A:1013193013142

Summary of study design

Expt 1: Druckery male rats (10/group) were orally dosed with 0, 4, 12, 20 mg Mo/kg bw/day [0, 10, 30 or 50 mg/kg/day SMD], 5 days/week for 60 days.

Expt 2: Druckery male rats (20/group) were orally dosed with 0 or 12 mg Mo/kg bw/day [0 or 30 mg/kg/day SMD], 5 days/week for 60 days and then mated to non-treated females

Endpoints measured:

- Molybdenum content of testes, epididymis, seminal vesicles
- Sperm count, motility and morphology
- Testicular enzymes: sorbitol dehydrogenase, lactate dehydrogenase, gamma glutamyl transpeptidase
- Organ weights: testes, epididymides, accessory sex organs
- Testicular histopathology (fixed in 10% neutral buffered formalin (NBF))
- Fertility parameters after mating to non-treated females (Expt 2)

Summary of reported results

- Increased levels of molybdenum in testis, epididymis and accessory organs compared with controls in 20 mg Mo/kg bw/d group
- No effects on testis weights (except relative weight, which is not relevant)
- Decreased epididymal weight at 12 and 20 mg Mo/kg bw/d, not dose related
- Decreased accessory sex organ weight at 12 and 20 mg Mo/kg/d
- Sperm count and sperm motility decreased and abnormal sperm morphology increased at 12 and 20 mg Mo/kg/d
- Decreased or increased levels of testicular enzymes at 12 and 20 mg Mo/kg/d
- Decreased fertility indices in Expt 2.

Major shortcomings of the study

- Druckery rats are an inbred strain, specific to ITRC Lucknow and not commonly used in regulatory toxicology studies
- It is unclear how many animals were used for the various endpoints measured. Although 10/group were dosed, some were sampled for molybdenum analysis, others for testicular enzymes and others for histopathology. The tables report that the number of animals for each endpoint was "*the requisite number of animals in each group*", but so few animals means that cannot be the case.
- Testes were fixed in 10% NBF and have major fixation artifacts precluding assessment of any changes
- Histopathology is the most important endpoint in this study but is poorly described. The single picture provided, is badly out of focus and it is impossible to say whether there is any cell degeneration. There is obvious increase in the interstitial space, but this is a common fixation artifact seen in tubules in the centre of the testis due to poor penetration of the fixative. There is no table providing any incidence or severity of

changes, nor how many animals were examined. This is essential since untreated rats can have incidental testicular findings in the testes, so to conclude there is a treatment related effect, one needs to see incidences and severity of any changes and evaluate any dose response. The text states that the 4 mg Mo/kg bw/d testes were almost normal but doesn't provide any indication of what, if any changes there were, and no incidences or severities of any findings are provided for the mid and high dose groups. Degeneration of interstitial cells would be a very rare and unlikely finding, and it would be expected to lead to a decrease in testosterone production, which would be reflected by atrophy of the seminal vesicles and prostate. There is a small decrease in absolute and/or relative seminal vesicle and prostate weight (not commented on by the authors), which could suggest that the high dose animals were subject to stress or body weight loss. Sluggishness was reported in the high dose group, but since no body weight or food intake data are provided, it is not possible to assess the condition of the animals.

- Sperm motility assessment was carried out using crude manual techniques inadequately described (1936 paper referenced). Sperm motility is a variable endpoint that requires carefully controlled methodology and is generally performed using computer assisted sperm analysis (CASA) with adequate Historical Control Data (HCD).
- Sperm count was measured using caudal sperm and should be reported as count /g of cauda. Instead it is presented per epididymis, which doesn't take into account the variable weight of tissue sampled.
- The decrease in some testicular enzymes (sorbitol dehydrogenase and gamma glutamyl transpeptidase) and the increase in lactate dehydrogenase are meaningless in the absence of any detailed histopathological changes.

Overall Comments

Although the overall design of this study is appropriate, the quality of the data generated is poor and unreliable. The overall conclusions regarding the effects of molybdenum on the testes and epididymis relies on the histopathology and sperm analysis, since these are the most sensitive endpoints for detecting testicular toxicity. However, the fixation of the testes is inadequate and the reporting and description of any findings in the testes is poor and incomplete. The methodology used to measure and report sperm parameters is also poor.

Murray, F.J., Sullivan, F.M., Tiwary, A.K., Carey, S., 2014. 90-Day subchronic toxicity study of sodium molybdate dihydrate in rats. Regulatory Toxicology and Pharmacology 70, 579–588. https://doi.org/10.1016/j.yrtph.2013.09.003

Summary of study design

Sodium molybdate dihydrate (SMD) was administered to Sprague Dawley rats (10/sex/group) in the diet to provide 0, 5, 17 and 60 mg Mo/kg bw/day [0, 12.5, 42.5 and 150 mg SMD/kg bw/day] for 90 days. Additional groups of rats (10/sex/group) dosed with 0 or 60 mg Mo/kg/day for 90 days were evaluated after a 60 day recovery period

Endpoints (relevant to male reproductive function) measured:

- Blood and serum concentrations of molybdenum during weeks 4 and 12 of dosing and during recovery

- Testicular spermatid head count, epididymal sperm count, total motility, progressive motility and sperm morphology
- Daily body weight, food intake, and clinical signs
- Organ weights of testes, epididymides, seminal vesicles and prostate (accessory sex organs)
- Histopathology of testes (fixed in Modified Davidsons), epididymides, and accessory sex organs

Summary of reported results

- Dose related increased levels of molybdenum present in serum compared with controls. No or little accumulation of molybdenum in the serum between weeks 4 and 12. Rapid decrease of serum molybdenum during week 1 of recovery.
- Decreased body weight and bodyweight gain in 60 mg Mo/kg bw/day rats compared with controls (15% less than controls)
- Reduced food intake and food conversion efficiency in 60 mg Mo/kg bw/day rats
- No treatment related effects on absolute organ weights of testes, epididymides or accessory sex organs but an increase in the relative weight of testes and epididymides of 60 mg Mo/kg/day group, which is expected, due to the body weight decrease.
- No effects on testicular or epididymal sperm count, total sperm motility or sperm morphology. A slight but statistically significant reduction in the number of progressively motile sperm was seen at 60 mg Mo/kg bw/day compared with controls (59% vs 69.4% in controls) but the mean values for progressive motility in the control group were at the upper limit of historical control values, whereas the progressive motility value for the 60 mg Mo/kg bw/day group (59.0%) was close to the historical control mean value (59.8 ± 16.2%). A lack of relationship to treatment was supported by the fact that there were no correlating reductions in total sperm motility in this study or in either generation of the 2 generation study (Murray 2019).
- No treatment-related histopathological findings in the testes, epididymides or accessory sex organs

Major shortcomings of the study

- None

Overall Comments

A significant advantage of this study compared with most of the other studies included in this review is that it was designed and performed in accordance with regulatory guidelines and performed under Good Laboratory Practice (GLP) guidelines in a GLP compliant laboratory (Huntingdon Life Sciences), who are very experienced in conducting this design of study. The study followed OECD Guideline 408 (**Repeated Dose 90-Day Oral Toxicity Study in Rodents)** but was supplemented with additional parameters (estrous cycles and sperm analyses), from OECD Guideline 416 (**Two-Generation Reproduction Toxicity).** *The advantage of following regulatory guidelines, is that it ensures adequate numbers of animals are used to provide statistically meaningful results, the parameters measured are those that are considered important to provide an overall assessment of toxicity and the methodology used for generating data for the different endpoints has been recommended and validated by the performing laboratory. Method validation is especially important when measuring quantitative parameters such as sperm analysis, serum analysis for the test article, clinical chemistry and hormone*

analysis. The methodology needs to have been validated and needs to be performed by suitably qualified and accredited individuals. GLP compliance of a study and a laboratory, provides assurance that such procedures are in place. In fact, I was the study pathologist for this particular study and so I had direct input into the histopathological evaluation and interpretation. Since this study was designed using regulatory guidelines and was GLP compliant, it (and the two-generation reproduction study) serve as the most convincing data sets with which to compare the results from the other investigative studies.

Based on the measured food intake and bodyweight of each dose group, the molybdenum test substance added to the diet was recalculated weekly to ensure the target dose was achieved (actual mean intakes over the treatment period were 0, 5.0, 17.1 and 60.0 mg Mo/kg bw/day]. Serum analysis for molybdenum demonstrated that molybdenum was absorbed from the diet and tissue analysis (liver and kidney) demonstrated dose related tissue exposure to molybdenum.

There were no treatment related organ weight changes for testes, epididymides or accessory sex organs except those associated with, and secondary to the significant body weight loss in the 60 mg Mo/kg bw/day group. The weight of most tissues are proportional to body weight and so if animals experience reduced body weight gain during a toxicity study, the absolute weight of most tissues would be expected to be reduced when compared with controls, but the body weight-relative organ weights would be expected to be the same as controls. This is not the case with testis weight, which like the brain, are maintained despite reduced body weight gain. This means relative weight of testes and to some extent, epididymides, usually increase when body weight gain decreases. So absolute weight needs to be used when evaluating testes and in this study it was unchanged when compared with controls, whereas relative weight increased as expected. It is also noteworthy that there was no decrease in the weight of the seminal vesicles and prostate, either absolute or relative to body weight. The accessory sex organs are a sensitive indicator of decreased androgen status and are more sensitive than histopathology, so the absence of any decrease in weight indicates normal levels of circulating testosterone.

Sperm analysis showed no treatment-related changes in sperm output from the testes (spermatid head count), sperm count in the cauda epididymis, sperm morphology, or in total sperm motility or progressively motile sperm. Based on the results of this study, the NOAEL for male reproductive toxicity was considered to be >60 mg Mo/kg bw/day while the overall NOAEL was 17 mg Mo/kg/day due to adverse effects on body weight at 60 mg Mo/kg bw/day.

Murray, F.J., Sullivan, F.M., Hubbard, S.A., Hoberman, A.M., Carey, S., 2019. A two-generation reproductive toxicity study of sodium molybdate dihydrate administered in drinking water or diet to Sprague-Dawley rats. Reproductive Toxicology 84, 75–92. https://doi.org/10.1016/j.reprotox.2018.11.004

Summary of study design

Groups of 24 male and 24 female Sprague-Dawley rats were administered sodium molybdate dihydrate (SMD) at 0, 5, 17, or 40 mg Mo/kg bw/day [0, 12.5, 42.6, 100 mg SMD/kg bw/day] in the drinking water or 40 mg Mo/kg/day in the diet over two generations. The parental generation and F1 generation were dosed for 10 weeks prior to mating and 90 days in total prior to necropsy. The F2 generation were exposed via lactation, up to weaning and then necropsied.

Endpoints (relevant to male reproductive function) measured:

- Blood/serum, urine, liver and kidney concentrations of molybdenum.
- Body weight, food and water intake, and clinical signs (P, F1 and F2 generations)
- Fertility parameters for P and F1 generations after ≥10 weeks dosing
- Testicular spermatid head count, epididymal sperm count, total motility, and sperm morphology after 90 days dosing (P and F1 generation)
- Organ weights of testes, epididymides, seminal vesicles and prostate after 90 days dosing (P and F1 generations)
- Histopathology of testes (fixed in Modified Davidsons), epididymides, seminal vesicles and prostate (P and F1 generations)

Summary of reported results

- Dose related levels of molybdenum present in serum liver and kidney. Greater levels of molybdenum were seen in the males administered 40 mg Mo/kg bw/d in the diet than in the drinking water although the range of weekly consumed doses was comparable at 35.2 – 46.1 mg Mo/kg bw/day for drinking water and 35.3 – 46.8 mg Mo/kg bw/day for diet in the P generation and 13.8-60.8 and 19.4 – 59.3 in the F1, respectively.
- Decreased body weight and bodyweight gain in P and F1 males given 40 mg Mo/kg bw/d in the diet (6-9% decrease compared with controls). There were no treatment related changes in body weight of males administered SMD in the drinking water
- No effect on food or water intake in P males administered SMD in diet or water but a decrease in food intake for F1 males administered 17 and 40 mg Mo/kg bw/d in drinking water and a decrease in food intake in males given 40 mg Mo/kg bw/d in the diet.
- No effects on any of the fertility parameters for P or F1 generation rats administered SMD in diet or drinking water up to 40 mg Mo/kg bw/d.
- A small (statistically significant) decrease in absolute organ weight of the epididymis in P rats administered 40 mg Mo/kg bw/day in the diet but this was not accompanied by a decrease in epididymal sperm content and was not accompanied by a decrease in body weight-relative epididymal weight. Absolute testis weight was also unaffected.
- No treatment related effects on organ weights of testes, epididymides or accessory sex organs.
- No treatment related effects on testicular or epididymal sperm count, sperm motility or sperm morphology in the P and F1 generations. Compared with controls, there was a slight but statistically significant increase in the number of sperm without a head in the P rats administered 40 mg Mo/kg bw/day in the diet. However, this was not observed in

the F1 generation rats given the same dose and the number of sperm in the P generation without a head was close to the average value in the historical control data base. Compared with controls, the total number of abnormal sperm versus normal sperm were similar.

- No treatment-related histopathological findings were observed in the testes, epididymides or accessory sex organs of the P or F1 rats

Major shortcomings of the study

- None

Overall Comments

As with the previous 90 day study performed by Murray 2014, this study was carried out in accordance with regulatory guidelines (OECD 416) in a GLP compliant laboratory that specialises in performing these complex studies. As such, this study provides the benchmark for quality of study design, methodology and presentation/reliability of a full data set. The study uses large group sizes of 24/sex/group and monitors toxicity through 2 generations incorporating in utero and lactation exposure to SMD as well as an additional >90 days postweaning exposure in the F1 generation. Dose levels were high enough to produce mild systemic toxicity in the form of body weight loss in the P and F1 generation and reduction in food intake and water intake in the F1 generation. Even at the highest dose level there were no treatment related changes in organ weight of testes, epididymides or accessory sex organs, no histopathological changes in these tissues, no changes in sperm parameters and no effects on fertility parameters.

Based on the results of this study the no-observed-adverse-effect level (NOAEL) for general toxicity was 17 mg Mo/kg bw/day based on reductions in body weight and food consumption in the 40 mg Mo/kg bw/day dose groups (diet and drinking water), whereas the NOAEL for males (and females) of the P and F1 generations for reproductive toxicity, including mating and fertility was 40 mg Mo/kg bw/day, whether exposure occurred in the diet or drinking water.

Zhai, X.-W., Zhang, Y.-L., Qi, Q., Bai, Y., Chen, X.-L., Jin, L.-J., Ma, X.-G., Shu, R.-Z., Yang, Z.-J., Liu, F.-J., 2013. Effects of molybdenum on sperm quality and testis oxidative stress. Syst Biol Reprod Med 59, 251–255. https://doi.org/10.3109/19396368.2013.791347

Summary of study design

SMD was administered to ICR strain of mice (10/group) in the drinking water at doses of 0, 12.5, 25, 50, 100 and 200 mg/L for 14 days. This is equivalent to 0, 2.25, 4.5, 9.0, 18.0, 36 mg SMD/kg bw/d or 0, 0.9, 1.8, 3.6, 7.2, 14.4 mg molybdenum/kg bw/day [conversion factor 0.18 for mouse subacute, EFSA 2012]

Endpoints measured:

- Relative (to bw) epididymis weight
- Sperm count, motility and morphology
- Testicular enzymes (MDA, superoxide dismutase, glutathione)

Summary of reported results

- Increase in epididymal weight, sperm count, sperm motility and decrease in abnormal sperm at 25 mg/L (1.8 mg Mo/kg bw/d)
- Decrease in epididymal weight, sperm count, sperm motility and increase in abnormal sperm at 100 and 200 mg/L (7.2 and 14.4 mg Mo/kg bw/d)
- Increase in testicular antioxidant enzymes at 12.5 and 25 mg/L (0.9 and 1.8 mg Mo/kg bw/d), decrease at >50 mg/L (>3.6 mg Mo/kg bw/d). MDA increased at 100 and 200 mg/L (7.2 and 14.4 mg Mo/kg bw/d)

Major shortcomings of the study

- Mice were administered a specific concentration of SMD in the drinking water but in the absence of water intake measurements or serum analysis of Mo levels, the intake of Mo can only be estimated. This makes it difficult to make comparisons between the results in this study and others.
- There is no mention of clinical condition, body weight, food intake or water intake being measured. The SMD in the drinking water may have been unpalatable at the higher concentrations. Testis weight was not measured and no histopathology was performed.
- The duration of dosing with SMD was 14 days, which is the approximate time taken for sperm leaving the testis to reach the cauda epididymis, which is where sperm are sampled. Therefore any sperm measurements relate to sperm that were exposed during transit through the epididymis. However, the authors relate the changes they saw in sperm parameters to putative effects of SMD on the testes.
- Mice become sexually mature at approximately 7 weeks of age. In this study the mice were only 3-4 weeks of age at the start of dosing, so presumably would have been 5-6 weeks of age at the end of dosing. This would make them peripubertal and therefore subject to wide variations in sperm content of the epididymis. Studies investigating male reproductive toxicity should be performed on sexually mature animals so the age of the animals in this study make the sperm data unreliable.
- The description of methodology for measuring sperm parameters and testicular enzymes is rudimentary and lacking any detail.
- The changes reported for sperm parameters and testicular enzymes lack a dose response, they appear to be improved at lower doses and decline at higher doses.

Overall Comments

A major problem with this study is the absence of any information on Mo intake by the mice and the sexual immaturity of the animals. There is no information on body weight gain, water intake or clinical condition of the mice and so it is unknown what condition the higher dose animals were in. If sperm parameters were affected by treatment then the duration of dosing would infer such changes were due to effects occurring in the epididymis and not the testes, so the conclusions of the study are not supported by the study design. The age of the mice would result in a lot of variability in sperm parameters because they would be in peripubertal status with some animals reaching sexual maturity ahead of others. This may explain the apparent improvement in sperm parameters in some groups compared with controls. It may also explain the extremely high rate of abnormal sperm in the control group (>28%) whereas the expected rate for ICR mice is 6.8% (https://www.criver.com/sites/default/files/noindex/historical-control-data/hcd-pa-mice.pdf).

For the above reasons, I consider this study design flawed and the results unreliable.

Wang, H.-W., Zhou, B., Zhang, S., Guo, H., Zhang, J., Zhao, J., Tian, E., 2016. Reproductive toxicity in male mice after exposure to high molybdenum and low copper concentrations. Toxicol Ind Health 32, 1598–1606. https://doi.org/10.1177/0748233715569269

Summary of study design

Molybdenum (not otherwise specified) was administered to Kunming mice in the drinking water at a concentration of 400 mg/L for 50 or 100 days. [Equivalent to 60 mg Mo/kg bw/day]. This was administered to mice (20 males/group) fed copper deficient diet supplemented with copper in the drinking water or copper deficient diet with no additional copper supplement. Groups of mice given the same copper supplemented or copper deficient diet served as controls. The copper deficient diet was non-standardised and prepared in the laboratory with a mixture of wheat, corn and soybean. Copper deficiency was achieved by using soybeans grown in copper deficient soil. Animals were killed after 50 or 100 days dosing.

Endpoints measured:

- Weekly changes in body weight up to Day 50 (6/group measured); terminated after 50 or 100 days
- Sperm count, sperm motility and morphology of sperm in whole epididymis
- Serum and testis levels of lipid peroxidation enzymes (MDA, SOD, GSHPx)
- Histopathology of testes (fixed in 4% paraformaldehyde in phosphate buffered saline)
- Ultrastructure of testes using transmission electron microscopy (TEM) (immersion fixed in 2.5% glutaraldehyde)

Summary of reported results

- No difference in final body weight at 50 days compared with controls; data after day 50 not reported
- Decrease in sperm count and motility and increase in abnormal sperm morphology in the low copper group, the low copper + Mo group and in the Mo and copper supplemented group
- Increased MDA and decreased SOD and GSHPx in the low copper group, the low copper + Mo group and in the Mo and copper supplemented group
- Histopathological and ultrastructural changes reported in groups treated with Mo and enhanced by copper deficiency

Major shortcomings of the study

- Mo was administered in drinking water but water intake was not measured so the dose level of Mo can only be estimated. Using the EFSA conversion factor of 0.15 would convert this dose level of 400 mg/L to 60 mg Mo/kg bw/d, but this would only apply if the material used was Mo and not one of the salts (and this is unspecified).
- Results are provided for serum biochemistry (total protein, albumin and blood urea nitrogen) but there is no mention of this being performed in the methodology
- There are no organ weights to support the changes reported in sperm count or the histopathological changes reported for the testes

- Sperm parameters were sampled from the whole epididymis. The normal procedure would be to sample from the cauda epididymis and vas deferens only, and express the results per g of tissue. Sperm in the rest of the epididymis are non-motile and immature so these would interfere with the measurements of the caudal sperm.
- The methodology for measuring sperm parameters is primitive, using a hemocytometer
- The histopathology was performed on paraformaldehyde fixed tissue, which has provided very poor fixation and artifact. Despite the authors claiming there is major disruption of spermatogenesis in the treated testes, all of the testes appear to suffer from major fixation artifact to the same degree, especially in the peripherally located tubules. I do not consider these few pictures, which are of poor quality, provide any evidence for treatment related histopathological changes. In addition, there is no information on incidence or severity of morphological changes.
- The methodology used for ultrastructural evaluation is also poor. Testes are very difficult to fix well for ultrastructural evaluation. The authors have used immersion fixation for electron microscopy of testes whereas cardiac or testicular perfusion fixation is the recommended methodology (Hess 1993, Creasy 2002). Although there are fixation artifacts in these pictures, the main problem lies in the lack of distinction between the different cell types within the germinal epithelium and no mention of the stage of tubule. The germinal epithelium is made up of many different cell types which all have very different ultrastructure. Some of the spermatogenic cells pictured are spermatocytes, some are round spermatids, others are elongating spermatids and others are largely made up of Sertoli cell cytoplasm. Each cell has very different mitochondrial characteristics with vacuolated mitochondria being a normal feature of pachytene spermatocytes. Lysosomes are also a normal feature of Sertoli cell cytoplasm, which forms processes between all the germ cells. I do not believe the photomicrographs in this manuscript illustrate any abnormalities that can be ascribed to treatment. They illustrate the normal features of the different cell types with some fixation artifact. I do not consider that the conclusions relating to marked vacuolization of mitochondria are supported by the TEM carried out.
- Enzymes relating to lipid peroxidation were measured in the serum and the testes. Approximately the same % changes from controls were reported for serum and testes. Lipid peroxidation is not a marker of injury and is only relevant if the changes are accompanied by evidence of morphological damage. I do not consider the authors have demonstrated any morphological damage in this study.

Overall Comments

The study design lacks any clear measurement of how much Mo the animals were exposed to or in what form. It also appears overly complex in feeding animals a non-standard diet containing soy beans grown in copper deficient soil and then making up for the copper deficit by adding additional copper to the drinking water. With regard to the evaluation of male reproductive parameters, there are no organ weights and there is negligible detail for the methodology used for sperm assessment, histopathology and TEM. What little information there is demonstrates that the methodology is very basic and uses inappropriate sampling techniques and fixatives. The description and photomicrographs of testicular histopathology and ultrastructure indicate that the authors have very limited knowledge of the normal features of the seminiferous tubules and their cell types. I do not consider the data in this study to be reliable.

Jeter, M.A., Davis, G.K., 1954. The effect of dietary molybdenum upon growth, hemoglobin, reproduction and lactation of rats. J Nutr 54, 215–220. https://doi.org/10.1093/jn/54.2.215

Summary of study design

SMD was administered to a Long-Evans strain of rats in the diet at a concentration of 0, 5, 20, 80 or 140ppm of the diet. Equivalent to 0, 1.8, 7.2 and 12.6 mg SMD/kg bw/day or 0, 0.72, 2.88 and 5.04 mg Mo/kg bw/day [subchronic rat conversion factor 0.09, EFSA 2012].

Varying concentrations of copper sulphate were also added to the diet to evaluate the effects of copper. Iron and manganese were also added part way through the dosing regime to enhance reproduction. Males and females (4/sex/group) were dosed from weaning to 11 weeks of age and then mated.

Endpoints measured:

- Weekly measurement of body weight (4 rats/sex/group) up to 11 weeks of age
- Food consumption was measured "periodically"
- Haemoglobin levels
- Fertility parameters
- Histopathology of testes from infertile males

Summary of reported results

- Decrease in body weight gain in rats fed molybdenum and low copper diet
- Decreased fertility in rats fed molybdenum at 80 and 140ppm and low copper
- Tubular degeneration of testes from infertile rats

Major shortcomings of the study

- The study fails to provide any details on methodology and very little data to support the conclusions
- Group sizes are very small and inadequate (only 4 rats/sex/group). Fertility assessments are currently performed using 20 rats/sex/group [OECD TG 443]
- Mo was administered in a poorly defined unconventional diet made up of mineralised milk sucrose mixture that was supplemented with copper, manganese and iron
- The dose levels of Mo are provided as ppm of the diet but the actual intake of Mo is not known since food intake was not measured, so it's not possible to compare dose levels in this study with other studies. However, using the EFSA subchronic rat conversion factor of 0.09, dose levels can be estimated as 0, 1.8, 7.2, and 12.6 mg SMD/kg/bw/day or 0, 0.72, 2.88. and 5.04 mg Mo/kg bw/day
- Histopathology was only performed on rats that proved infertile (a total of 6 rats) and the description of changes is perfunctory

Overall Comments

The study is very old (1954) and the methodology is poorly if at all described. Group sizes of 4/sex/group are very small and inadequate to assess fertility parameters. With this proviso the 4 males fed Mo at 80ppm plus copper supplement were all fertile compared with only 1 of 4 males fed 80ppm Mo in a copper deficient diet that was fertile.

Khorami, H., Eidi, A., Mortazavi, P., Modaresi, M., 2020. Effect of sodium molybdate on cadmium-related testicular damage in adult male Wistar rats. Journal of Trace Elements in Medicine and Biology 62, 126621. https://doi.org/10.1016/j.jtemb.2020.126621

Summary of study design

Sodium molybdate was administered by oral gavage to male Wistar rats (6 males/group) at 0, 0.02, 0.04, 0.08 and 0.16 mg Mo/kg bw/d [0, 0.05, 0.1, 0.2 and 0.4 mg SMD/kg bw/d] for 30 days. This dosing regime was performed on otherwise healthy rats or in conjunction with I.P. administration of 3mg/kg/d of cadmium chloride ($CdCl_2$), a known potent testicular toxicant in rats. The purpose of the study was to investigate any effects of Mo on testicular function and to investigate whether Mo is protective against the adverse effects of $CdCl_2$ on testicular function.

Endpoints measured:

- Sperm parameters (count, motility, viability, morphology, membrane integrity)
- Testicular lipid peroxidation/ oxidative stress enzymes (MDA, SOD, catalase (CAT), glutathione peroxidase (GPX)
- Histopathology of testes (fixed in Bouin's), using semi quantitative assessment
- Immunohistochemistry for Aquaporin 9 (AQP9)

Summary of reported results

- SMD had no effect on any of the sperm parameters at doses up to 0.16 mg Mo/kg bw/d in healthy rats
- SMD had no effects on any of the lipid peroxidation/oxidative stress enzymes at doses up to 0.16 mg Mo/kg bw/d
- SMD had no effects on testicular histopathology or AQP9 distribution at doses up to 0.16 mg Mo/kg bw/d
- SMD at all doses reduced the adverse effects of $CdCl_2$ on sperm parameters
- SMD at 0.08 and 0.16 mg Mo/kg bw/d attenuated the changes in MDA, SOD, CAT and GPX resulting from $CdCl_2$ administration
- SMD at all doses reduced the severity of histopathological damage caused by $CdCl_2$

Major shortcomings of the study

- The entire epididymis appears to have been used to obtain sperm for analysis whereas the cauda and vas deferens are routinely used.

Overall Comments

The study appears to be well carried out. CASA was used to assess sperm motility and Bouin's fixative was used to fix the testes. Although the photomicrographs of testicular histopathology lack resolution, they are adequate to demonstrate the normal structure of the SM treated testes and the significant changes in the $CdCl_2$-treated testes. This manuscript illustrates tubules that are in the same spermatogenic stage (Stage VII/VIII), except where the tubules are so damaged that the stage cannot be identified. Histopathological changes have been assessed using a semi-quantitative scoring system (Johnson's score) and the data tabulated (Table 4). Most other studies reviewed here fail to provide any incidence or severity data for the conclusions drawn.

The study demonstrates that gavage dosing with SMD at dose levels of up to 0.4 mg Mo/kg bw/d for 30 days has no effects on sperm parameters, histopathology or lipid peroxidation/oxidative enzymes of the testis and conversely, improves these parameters in rats dosed with the potent testicular toxicant CdCl2. The doses of SMD used in this study are below those used by Pandey (2002) who also used oral gavage administration, making it difficult to make any direct comparisons.

NTP, 1997. NTP Toxicology and Carcinogenesis Studies of Molybdenum Trioxide (CAS No. 1313-27-5) in F344 Rats and B6C3F1 Mice (Inhalation Studies). Natl Toxicol Program Tech Rep Ser 462, 1–269.

Summary of study design

F344 rats and B6C3F1 mice were dosed with molybdenum trioxide (MoO_3) by inhalation (6hr/day, 5 days/week) for 13 weeks (10/sex/group). Doses were 0, 3, 10, 30 and 100 mg MoO_3/m^3, equivalent to 2, 7, 20 and 67 mg Mo/ m^3

The study also included subsets of rats and mice dosed for 2 and 104 weeks but the main endpoints measured (nasal histopathology in the 2 week study and tumor incidence in the 104 week study) are not relevant to this review. However, blood molybdenum levels were measured in the chronic duration 104 week study, which provides useful information for comparison with other studies.

Endpoints measured:

- Body weight and organ weights,
- Sperm parameters (testicular spermatid head count, epididymal sperm count and motility
- Histopathology of reproductive tissues (testes and epididymides fixed in Bouin's)
- Blood levels of molybdenum (sampled in the 104 week study)

Summary of reported results

- MoO_3 had no effect on body weight gain of male mice or rats over 13 weeks exposure.
- There were no treatment related changes in testicular spermatid count or in epididymal sperm count or sperm motility in either species.
- There were no treatment related changes in organ weights of either species
- There were no treatment related histopathological changes in the reproductive tissues of either species
- Mo levels showed an exposure related increase in the blood of treated rats and mice (sampled in the 104 week study) . In rats, the blood levels of Mo were: 221ng/mL (0 mg MoO_3/m^3), 800 ng/mL (10 mg MoO_3/m^3), 1774 ng/mL (30 mg MoO_3/m^3), 6036 ng/mL (100 mg MoO_3/m^3). For comparison purposes, the blood levels of Mo measured at the high dose (100 mg MoO_3/m^3) in this inhalation study are equivalent to the blood levels measured in rats administered 20 mg Mo/kg bw in the diet.

Major shortcomings of the study

- None

Overall Comments

Toxicity studies performed as part of the National Toxicology Program (NTP) are carried out to a standardised protocol and to a high quality, similar to those carried out in accordance with regulatory guidelines. The methodology they use is standardized and validated and they have an extensive database of historical control data as well as vast experience of performing and evaluating these routine studies. As such, I would consider the data generated from these studies and the overall evaluation of molybdenum toxicity as being very reliable.

Conclusions

In this review, I have concentrated on studies that have set out to investigate the effects of molybdenum on the male reproductive system. Some studies reported adverse effects on testicular histopathology, sperm parameters or testicular enzymes (Jeter 1954, Pandey 2002, , Zhai 2013, Wang 2016, while others reported no treatment related effects on any male reproductive endpoints (Murray 2014, 2019, NTP 1997). One study even reported that molybdenum had a protective effect on CdCl2 induced testicular injury (Khorami 2020)

Having reviewed the study design, methodology, generated data and conclusions of the molybdenum toxicity studies included in this review, there are three studies that stand out from the rest with respect to quality, completeness and reliability of data. They are the studies that were performed in GLP compliant laboratories and in accordance with regulatory guidelines (**Murray 2014, 2019**) and the **study performed by the National Toxicology Program**, which was also performed to strict NTP guidelines by a laboratory very experienced in conducting and evaluating these types of studies. **All three studies, failed to demonstrate any adverse effects on male reproductive morphology or function at the dose levels administered**.

As outlined in this review, accurate evaluation of the male reproductive system requires considerable expertise and experience. This is especially true for histopathological and ultrastructural evaluation of the testes. With only two exceptions, the investigative studies that claimed histopathological abnormalities related to molybdenum administration, failed to provide adequate evidence of the changes they described and in most cases the illustrations provided, demonstrated pronounced fixation and processing artifacts or misinterpretation of features, based on comparing tubules in different stages of the spermatogenic cycle. The absence of any data relating to incidence or severity of histopathological findings precludes any assessment of dose response or relationship to molybdenum exposure.

The other study that provided incidence and severity data as well as acceptable evidence of histopathologic changes was the study of Khorami 2020. This study used a semi quantitative assessment (Johnson's score) to score the incidence and degree of damage in the seminiferous tubules. They reported an absence of any testicular findings when molybdate was administered on its own, but reported an improvement in the Johnson's score when molybdenum was co-administered with the testicular toxicant CdCl2 (compared with CdCl2 administration on its own).

The GLP studies performed to OECD regulatory/NTP guidelines (NTP 1997, Murray 2014, 2019) where group sizes were 10-24 rats/group and sperm parameters were measured using sensitive CASA technology did not show any treatment related effects caused by molybdenum. In contrast, most investigative studies that measured sperm parameters (count, motility and morphology), frequently using inadequate numbers of animals (generally <10) as well as poorly described and primitive methodology, concluded that molybdenum exposure resulted in decreased sperm count and motility and an increase in abnormal sperm at higher doses. One exception (Zhai 2013) found sperm parameters were increased/improved at lower doses of molybdenum compared with controls and Khorami 2020 found no effects of molybdenum on sperm parameters in healthy rats and improved sperm parameters in CdCl2 treated rats that were co-administered molybdenum.

As with any comparison of dietary and gavage administration, it is likely that the route of dosing along with the dose level of molybdenum administered would have influenced the toxicokinetic profile of molybdenum, providing higher but transient Cmax values with oral gavage than would be achieved by administration in the diet or drinking water. However, toxicokinetic data from a 28 day repeat dose study in the rat comparing dietary and gavage administration of SMD at 20 mg Mo/kg bw/day reported similar values for $AUC_{0\text{-}24h}$ for both routes (Khazaeinia 2011).

Blood levels of molybdenum were measured in the studies performed to OECD/NTP guidelines but they were not measured in any of the other studies, so a direct comparison of molybdenum exposure levels between studies is not feasible.

A number of the studies that reported decreased sperm parameters also modified the copper content of the diet either decreasing it or increasing it in conjunction with molybdenum. The rationale for this is based on the fact that features of molybdenum toxicity resemble those of copper deficiency and copper supplementation can alleviate the effects of molybdenum toxicity. In the studies performed by Murray (2014, 2019) using certified rodent diets (Harlan Teklad 2016C, PMI Nutrition International #5002), they demonstrated that administration of Mo resulted in increased copper levels in whole blood, serum and a variety of tissues, and theorised that the interaction between copper and molybdenum may represent an adaptive/protective response against toxicity. However, the investigative studies that added increased or decreased copper intake as a variable in the experimental design, in conjunction with administration of molybdenum, make direct comparison of molybdenum-mediated effects more difficult.

Whatever the reason for the discrepancies between the results of the studies, whether it be down to poor study design and methodology or due to different routes of administration and dose levels, or different strains of rats/mice, **I would suggest the following**:

1. Since human exposure to Mo occurs mainly through the diet and drinking water rather than an ingested bolus dose or an IP injection, **the studies using dietary or drinking water administration are more relevant for hazard identification in the general population** .
2. The toxicity studies that have been performed according to OECD/NTP guidelines in GLP compliant laboratories (**NTP 1997, Murray 2014, 2019) stand out as providing the most reliable and statistically robust data on which to base any hazard identification of molybdenum/compounds**.
3. Based on my critique of most of the investigative studies of molybdenum toxicity included in this review, **I consider the data unreliable and the conclusions unsupported**.

[redacted] 21 Nov 2024

Dianne M Creasy, Ph.D, Dip RCPath (tox), FRCPath. Date

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APPENDIX 1

First Author	Year	Species	Test item	Dose levels (mg Mo/kg bw/day)*	Route of administration	Duration of Exposure	Endpoints measured/reported	Testis Fixative
Murray	2014	Sprague Dawley rat	sodium molybdate dihydrate	0, 5, 17, 60	Diet	90 days exposure with 60 day recovery period	HRS, sperm count, motility and morphology, body weight, food intake, organ weights, histopathology. Elemental analysis of blood, tissues and feed	Modified Davidsons
Murray	2019	Sprague Dawley rat	sodium molybdate dihydrate	0, 5, 17, 40	Drinking water (5, 17, 40 mg/kg) Diet (40mg/kg)	10 wks prior to mating, 90 days total (Parental and F1 generation) . F2 generation through to weaning	Fertility parameters (after 10 weeks dosing), for P and F1,	Modified Davidsons
Pandey	2002	Druckery rat	sodium molybdate	0, 4, 12, 20 (Expt 1) 0, 12 (Expt 2)	Oral Gavage	5 days/week for 60 days	Sperm count, motility and morphology, body weight, organ weights, histopathology, fertility parameters, testicular enzyme analysis, molybdenum analysis of tissues	10% neutral buffered formalin
Zhai	2013	Mouse ICR strain	sodium molybdate dihydrate	0, 0.9, 1.8, 3.6, 7.2, 14.4	Drinking water	14 days	Testicular enzymes, sperm concentration, sperm motility, sperm morphology	
Jeter	1954	Long Evans rat	sodium molybdate dihydrate with varying levels of copper	0, 0.72, 2.88, 5.04	Diet	From weaning to 11 weeks of age	Body weight, fertility parameters, limited histopathology of testes	Not stated
Khorami	2020	Wistar rats	sodium molybdate with or without CdCl2	0, 0.02, 0.04, 0.08, 0.16	Oral gavage	30 days	Histopathology, testicular enzymes, sperm count, sperm motility, sperm morphology, sperm viability. AQP9 immunohistochemistery	Bouins
NTP	1997	F344 rats and B6C3F1 mice	Molybdenum trioxide	0, 3, 10, 30, 100 mg MoO_3/m^3	Inhalation	2, 13, and 104 week studies dosing 6hrs/day, 5 days/week	Body weight, food intake, organ weights, histopathology, sperm count, motility, morphology (after 13 weeks dosing), carcinogenicity (after 104 wk dosing)	Bouins
Wang	2015	Kunming mice	Molybdenum (not otherwise specified) with low and high copper containing diets	0, 60	Drinking water	Up to 100 days	Serum and testicular enzymes, sperm count, sperm motility and morphology, histopathology and ultrastructure of testes using transmission electron microscopy	4% paraformaldehyde

* Dose levels of Mo/kg bw/day calculated using a conversion factor based on a (rounded) 40% Mo content of SMD and using the "Factors for converting chemical substance concentrations in feed or drinking water into daily doses in experimental animal studies" [EFSA Scientific Committee; Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579. p 3.] doi:10.2903/j.efsa.2012.2579

Page/ Line No	DECOS content IMOA are commenting on:	IMOA Comment:
Executive Summary		
8/5	*Animal studies did indicate adverse effects on sperm count and quality in male rats and mice, although no conclusion could be drawn on whether exposure affected functional fertility*	The Expert Review of reported adverse male reproductive effects by a renowned expert in male reproductive histopathology, Dr Dianne Creasy, concludes that the data on sperm count and quality from most non-GLP, non-Guideline studies (with the exception of Khorami 2022) in rats and mice are unreliable and unsuitable for classification purposes owing to deficiencies in methodology and reporting. Furthermore, reliable, GLP compliant OECD/NTP Guideline studies at similar dose/exposure levels have not identified any adverse effects on male reproductive organs (5 studies, rats and mice: NTP 1997 (2 species), Murray 2014, 2019, IMOA 2016), seminology (4 studies, rat: NTP 1997, Murray 2014, 2019, Hoberman 2016) or functional fertility (2 studies, rat: Murray 2019, Hoberman 2016). **The collective weight of evidence in the Expert Review and in the cited multiple peer-reviewed publications mean classification as a suspected human reproductive toxicant is not justified.**
8/21-24	*Based on the available scientific data, the committee recommends:* • *to classify molybdenum and selected inorganic molybdenum compounds as suspected to be a reproductive toxicant to humans, which corresponds with category 2 for reproduction, and to label molybdenum with H361f (suspected of damaging fertility);*	The Expert Review of reported adverse male reproductive effects by a renowned expert in male reproductive histopathology, Dr Dianne Creasy, concludes that the data on sperm count and quality from most non-GLP, non-Guideline studies (with the exception of Khorami 2022) in rats and mice are unreliable and unsuitable for classification purposes owing to deficiencies in methodology and reporting. Furthermore, reliable, GLP compliant OECD/NTP Guideline studies at higher dose/exposure levels and with greater statistical power have not identified any adverse effects on male reproductive organs (5 studies, rats and mice: NTP 1997 (2 species), Murray 2014, 2019, IMOA 2016), seminology (4 studies, rat: NTP 1997, Murray 2014, 2019, Hoberman 2016) or functional fertility (2 studies, rat: Murray 2019, Hoberman 2016). **The collective weight of evidence in the Expert Review and in the cited multiple peer-reviewed publications mean classification as a suspected human reproductive toxicant is not justified.** OECD 2008. Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment. OECD Series on Testing and Assessment (No. 43), Organisation for Economic Cooperation and Development, Paris

		OECD 2013; Guidance document supporting OECD test Guideline 443 on the Extended One Generation Reproductive Toxicity Test. Series on Testing and Assessment No. 151. ENV/JM/MONO (2013)10 US EPA; 2024: Guidelines for Reproductive Toxicity Risk Assessment https://www.epa.gov/risk/guidelines-developmental-toxicity-risk-assessment Healey GF. Power Calculations in Toxicology. Alternatives to Laboratory Animals. 1987;15(2):132-139. doi:10.1177/026119298701500205 Channon EJ, Ashley AH. Power Calculations for General Toxicology Studies. Drug Information Journal. 1997;31(2):449-457. doi:10.1177/009286159703100215 Marieke S Jansen, Rolf H H Groenwold, Olaf M Dekkers, The power of sample size calculations, European Journal of Endocrinology, Volume 191, Issue 5, November 2024, Pages E5–E9, https://doi.org/10.1093/ejendo/lvae129 Kimmel CA, Gaylor DW. Issues in qualitative and quantitative risk analysis for developmental toxicology. Risk Anal. 1988 Mar;8(1):15-20. doi: 10.1111/j.1539-6924.1988.tb01149.x. PMID: 3375503. Jacobson-Kram, D., & Keller, K.A. (Eds.). (2006). Toxicological Testing Handbook: Principles, Applications and Data Interpretation (2nd ed.). CRC Press. https://doi.org/10.1201/b14280
2. Identity of the substance		
13/8 table 1	*From this list, a selection of compounds with available reproductive toxicity data was made, which included: **molybdenum**, sodium molybdate, ammonium molybdate (VI), and molybdenum trioxide (Table 1).*	IMOA is not aware of reproductive toxicity data/studies on the substance "molybdenum", i.e. the metallic / elemental form. This substance should not be listed in a table showing "molybdenum compounds with available reproductive toxicity data". See also comment on page 13, line 12.
13/8-9 tables 1/2	*From this list, a selection of compounds with available reproductive toxicity data was made, which included: molybdenum, sodium*	"ammonium molybdate (VI)" is an ambiguous substance description and should not be used. Molybdate and ammonia can form salts with various stoichiometries, and anhydrous and hydrated forms exist. Throughout the scientific literature this causes confusion or ambiguity.

molybdate, ***ammonium molybdate (VI)****, and molybdenum trioxide (Table 1).*

Often, it does not become clear to which substance exactly the authors refer to when using the term “ammonium molybdate”. Including the Mo-oxidation state, as in “ammonium molybdate (VI)” is also not clearly describing one defined substance. “Ammonium molybdate” may even be sometimes confusingly used for ammonium phosphomolybdate, $(NH_4)_3PMo_{12}O_{40}$ or ammonium tetrathiomolybdate, $(NH_4)_2MoS_4$.
The commercially relevant substances are the following three, and the names from this table should be used. All three are EU REACH registered by their respective Lead Registrants under the auspices of The IMOA EU REACH Molybdenum Consortium (MoCon).

Correct name	**Formula**	**CAS**	**EC No**
hexaammonium heptamolybdate (tetrahydrate) also: ammonium heptamolybdate (AHM)	$(NH_4)_6Mo_7O_{24}$	12027-67-7 (anhydrous) 12054-85-2 (tetrahydrate)	234-722-4
tetraammonium octamolybdate* also: ammonium octamolybdate (AOM)	$(NH_4)_4Mo_8O_{26}$	12411-64-2	235-650-6
diammonium dimolybdate also: ammonium dimolybdate (ADM)	$(NH_4)_2Mo_2O_7$	27546-07-2	248-517-2

* The EC Name for this substance erroneously is “tetraammonium *hexa*molybdate”, which is very misleading. The correct name is “tetraammonium octamolybdate”. This, or simply ammonium octamolybdate, abbreviated to “AOM”, are the names used by industry for this substance. It is a possibility that the mistake in the EC inventory originates from the full systematic name of the substance, $(NH_4)_4Mo_8O_{26}$, which is tetraammonium hexacosaoxo octamolybdate. The hexacosaoxo indicates the 26 (hexacosa) oxygen atoms in the formula $(NH_4)_4Mo_8O_{26}$. At some point in the history of the EC inventory, the hexacosaoxo possibly became erroneously truncated to only hexa and the octa from octamolybdate was omitted.

Ambiguous names, such as “ammonium molybdate” or “ammonium molybdate (VI)” should not be used.

Other unclear / misleading / outdated identifiers:

EC	**CAS**	**EC Name**	**Remark**
236-031-3	13106-76-8	ammonium molybdate(VI)	One REACH Registrant unknown to IMOA. Ceases manufacture in 2023. Apparent formula $(NH_4)_2MoO_4$.
601-720-3	12054-85-2	azanium; molybdenum;	The CAS entry for 12054-85-2 indicates that this is ammonium heptamolybdate *tetrahydrate.* Under EU REACH, the tetrahydrate is registered under the same EC number as the

<table>
<tr><td></td><td></td><td>
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<tr><td></td><td></td><td>oxygen(2-); hydrate</td><td>anhydrous form. Therefore, the EC number 601-720-3 is obsolete.</td></tr>
<tr><td>603-021-9</td><td>12501-45-0</td><td>Molybdate (Mo7O246-), ammonium (1:6)</td><td>This is likely an old/redundant entry in the EC inventory for hexaammonium heptamolybdate.</td></tr>
</table>
</td></tr>
<tr><td>13/12-14</td><td>The group approach is based on the ECHAs Read-Across Assessment Framework of metal compounds (according to RAAF Scenario 3)</td><td>IMOA agrees in principle with the read-across / grouping concept being based on the molybdate ion (RAAF Scenario 3), as this is in line with the approach that the IMOA EU REACH Molybdenum Consortium has taken for the REACH registrations. However, DECOS rightfully notes that "systemic toxicity correlates with the ability of the substance to release molybdate ions". There are several poorly soluble / insoluble molybdenum substances on the market (and used at workplaces), such as the metal itself (on its own or in alloys), and also molybdenum disulfide. Any hazard classification assessment should clearly and unambiguously distinguish between those substances within scope, and those poorly soluble/insoluble substances which are out of scope. When assessing hazard classification, which can have substantial downstream consequences, there should be an unambiguous list of substances that are concerned.</td></tr>
<tr><td>15/Table 1</td><td>footnote a to table 1
Table 1. Substance identity and information related to molecular and structural formula of molybdenum compounds with available reproductive toxicity data: molybdenum, ammonium molybdate and disodium molybdate (dihydrate), and molybdenum trioxide.</td><td>This footnote a seems to refer to the substance "molybdenum sulfide (MoS2), roasted", EC 289-178-0, CAS 86089-09-0. This substance is not mentioned in table 1 at all and the reference letter does not appear on the table.</td></tr>
<tr><td>16/Table 2</td><td>Table 2. Substance identity and information related to molecular and structural formula of molybdenum compounds without available reproductive toxicity data: ammonium paramolybdate, diammonium dimolybdate, ammonium tetrathio molybdate, dipotassium tetraoxomolybdate, tetraammonium hexamolybdate, and silicon(4+) trioxomolybdenum dioxidandiide.</td><td>Neither ammonium tetrathiomolybdate, nor other tetrathiomolybdates (TTM) should be included in the "molybdate" read-across/category. The chemistry and biology of thiomolybdates is different from that of oxomolybdates. Since TTM is a potent copper chelator and a molybdenum-sulfur compound that behaves differently from molybdenum-oxygen compounds, it is not representative of the soluble molybdate releasing compounds that form the category.</td></tr>
</table>

19/Table 3	*Table 3. Summary of physicochemical properties of molybdenum and its selected compounds.*	Substance names used in table 3 are partly ambiguous e.g. ammonium molybdate(VI), ammonium paramolybdate. Thiomolybdates should not be in the category. For physico-chemical data like density or solubility for salts, it should be made clear if the data refers to anhydrous or hydrated forms.
2.3 International classifications		
21/17-18	*molybdenum trioxide has been classified in carcinogen category 3B by the German Research Foundation (DFG).*	There is no carcinogen category 3B by German DFG/MAK. It is category 3 and is specifically limited to molybdenum trioxide. Category 3 is defined as follows: "Substances that cause concern that they could be carcinogenic for man but cannot be assessed conclusively because of lack of data. The classification in Category 3 is provisional. Substances for which the available studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories."
21/29-31 22/1-3 Table 4	*In Japan, several classifications including classifications for reproductive toxicity are applicable for the selected molybdenum and selected inorganic molybdenum compounds (Table 4). Classifications for reproductive/ developmental toxicity were based on data available for sodium molybdate and, by means of read-across based on solubility, these classifications were extrapolated to ammonium (para)molybdate and molybdenum trioxide.*	This classification was derived by the Japanese National Institute of Technology and Evaluation (NITE) in 2015, based on earlier internal evaluations dated 2012, as documented on the NITE website. **The more recent GLP OECD Guideline studies were not available to NITE at the time of the assessment** but were submitted by IMOA in 2022 for consideration in future reviews of the classification. IMOA disagrees with several classifications proposed by NITE, as they are not supported by recent reliable data. Specifically on reproductive toxicity: As noted by DECOS, NITE has classified sodium molybdate as “Repr. 2 (H361: Suspected of damaging fertility or the unborn child)”. IMOA has reviewed the NITE rationale, including their 2012 report, and identified the studies that formed the basis for NITE to propose classification. NITE refers to only the three studies by Fungwe et al (1990), Jeter and Davis (1954), and Schroeder and Mitchner (1971). As detailed elsewhere in our comments, all three studies are low quality unreliable ones whose results were not reproducible in more recent, OECD guideline-compliant GLP studies (not available to NITE at the time of the assessment). Furthermore, NITE also noted that reported reproductive/developmental effects [in those older studies] occurred only at doses where general toxicity was observed in parent animals, suggesting that the effects on reproduction/development were likely secondary to the general toxicity in the parent animals. However, importantly, as per CLP regulation Annex VI, 3.7.2.2.1, "classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific

		property to produce an adverse effect on reproduction and substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects."
22/Table 4		See above on unambiguous substance identification, specifically on the ammonium molybdates. Thiomolybdates should not be considered.
3. Manufacture and Uses		
24/26-27	*The leading form of molybdenum used by industry, particularly in stainless steel production, is molybdenum trioxide.*	The form of "molybdenum trioxide", also known as "technical grade molybdenum oxide, CAS 86089-09-0, EC No. 289-178-0." is the one used in stainless steel production, as distinct from (pure) molybdenum trioxide CAS No. 1313-27-5, EC No. 215-204-7.
4. Toxicokinetics		
26/27	*Molybdenum in a dissolved state is taken up into the bloodstream, and the absorption rate is dependent on its solubility. The ICRP (2012) classified molybdenum sulfide, oxides and hydroxides as having a "slow" absorption*	This is cited from ATSDR, but there is an apparent error in the primary source ICRP (2012), as molybdenum hydroxide(s) are not known by IMOA/MoCon to exist. This should not be perpetuated by it being repeated. There are very few, probably erroneous mentions on the Internet of the substance "molybdenum hydroxide", CAS 126853-99-4. However, the official CAS Registry also classifies this compound as a "Tabular Inorganic Substance" (TIS)". According to CAS Definition, these TIS are inorganic compounds that do not receive a structure-based atom level connection table representing the entire material because one of the following is true: Its structure is unknown. It does not exist as a discrete molecule.
26/28	*This classification supports an expected terminal absorption half-time of around 19 years.*	The "terminal absorption half-time of around 19 years" is not a substance specific value for "molydenum sulfide, oxides and hydroxides" (see above comment on the hydroxide). Again, this is cited from ATSDR, but the "ca 19 years" are the 7000 days that ICRP uses as a default absorption half-live parameter in their Human Respiratory Tract Model (HRTM) in the absence of substance specific data for substances classified as "slow" or "Type S" for absorption in the respiratory tract. IMOA suggests removing the sentence containing the "19 years" to avoid perpetuating the misconception that this is a substance-specific value for said molybdenum compounds.
28/16-21	*Dermal Uptake*	'Skin membrane' is a somewhat odd phrase. IMOA suggests using "full thickness human skin samples" as in the original study report, which also clarifies that this was in fact *human* skin

		(neither from animals, nor a skin model system) and to add that the doses of 105 and 542 µg/cm² of the test item sodium molybdate dihydrate correspond to *ca.* 40 and 220 µg Mo/cm².
30/10-13	*The blood half-life for molybdenum may vary from several hours in laboratory animals up to several weeks in humans.* **Barceloux DG and Barceloux D. Molybdenum. Journal of Toxicology: Clinical Toxicology 1999; 37(2): 231-7.**	This statement is not supported by the reference or data. It appears to be a citation from DECOS Ref 29, Barceloux (1999). The relevant sentence in Barceloux (1999, page 235) reads: "Elimination is usually complete within several weeks". It is part of only a brief review paragraph by Barceloux about elimination. There is no reference given by Barceloux to a primary source for this statement so that it is not even clear if it refers to humans or experimental animals. Furthermore, "elimination is complete within several weeks" is not the same as "a half-life of several weeks". In first-order kinetics, ca. 5 half-lives are needed for 97% elimination, and elimination is generally considered as "complete" after 10 half-lives.
5. Mechanism of action and toxicity		
31/11-13	*There are indications that altered copper utilization is a significant factor, as demonstrated by studies that show more severe effects in copper-deficient animals*.	Specifically regarding reproductive and developmental toxicity of molybdate, IMOA highlight this recent open access publication on a GLP-compliant study designed to closely match that of the Fungwe et al. (1990) investigations, and thus explicitly using a marginal-copper diet [Murray et al 2023]. None of the reported findings by Fungwe 1990 were reproducible and therefore IMOA recommends that DECOS adjust the text on page 31 to either omit the statement, or reflect the Murray 2023 study, not least by writing e.g. 'There are **conflicting** indications [emphasis added]…'. F. J. Murray, L. Aveyard, S. A. Hubbard, A. M. Hoberman, and S. Carey, "Sodium molybdate dihydrate does not exhibit developmental or reproductive toxicity in Sprague-Dawley rats maintained on a marginal copper diet". Reproductive Toxicology, p. 108442, Jul. 2023, doi: 10.1016/j.reprotox.2023.108442.
31/29 -33 32/1-9	Paragraph beginning *'Several studies reported that molybdenum can induce oxidative stress* *Zhai X-W, Zhang Y-L, Qi Q, Bai Y, Chen X-L, Jin L-J, et al. 2013. Effects of molybdenum on sperm quality and testis oxidative stress. Systems biology in reproductive medicine 2013; 59(5): 251-5.*	This paragraph about "oxidative stress" merely cites four references (one on cell cultures, two on mice and one epidemiological) on a complex topic. Further comments are provided further below in this table and in the attached Expert Review by Dr. Creasy. Briefly: Markers of oxidative stress including malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx) superoxide dismutase (SOD) etc) have been postulated as indicators of testicular toxicity (Pandey 2002, Zhai 2013, Zhang 2013, Wang 2016, Khorami 2020) but these are not routine endpoints for detecting testicular toxicity, and are not recommended in any regulatory

	Zhang Y-L, Liu F-J, Chen X-L, Zhang Z-Q, Shu R-Z, Yu X-L, et al. 2013. Dual effects of molybdenum on mouse oocyte quality and ovarian oxidative stress. Systems biology in 12 reproductive medicine; 59(6): 312-8.	guidelines. While they can be useful adjuncts for investigating possible mechanisms of toxicity, they are inadequate when used on their own, because it is essential that any changes in enzyme levels can be shown to correlate with morphological or functional injury. The change is only toxicologically important if it is accompanied by good evidence of morphological or functional injury, indicating that the protective enzyme responses have been exceeded. Apart from this, the paragraph by DECOS does not consider the fact that some cited studies are of poor reliability and that other research has shown contradictory results: For example, the mice studies by Zhai et al. and Zhang et. al. (both 2013, DECOS references 67+68) both have major limitations including but not limited to the lack of any mention of validation for the assay methodology or of the kit used, which is an important prerequisite for such measurements [Griffiths 2002, Collins 2005.] A recent epidemiological paper Joun et al. [2024]), based on >15,000 participants in the USA, found that molybdenum provides antioxidative benefits and that increased urinary Mo is associated with lower levels of both systemic inflammation and oxidative stress. This is in contrast to Domingo-Relloso et al [2019], cited by DECOS as Ref 66., who did not focus solely on Mo but on various elements. Also, the association of Mo with oxidative stress in Domingo-Relloso [2019] was significant in only one of several different statistical models. IMOA recommends that DECOS reflects in this paragraph that (i) markers of oxidative stress on their own are not indicators of testicular toxicity, (ii) the limitation of cited studies and (iii) conflicting results from other research. Joun JH, Li L, An JN, et al. 2024. Antioxidative effects of molybdenum and its association with reduced prevalence of hyperuricemia in the adult population. PLoS ONE; 19(8): e0306025. doi: 10.1371/journal.pone.0306025 Griffiths HR, Møller L, Bartosz G, Bast A, et al. 2002. Biomarkers. Molecular Aspects of Medicine Volume 23, Issues 1–3, Pages 101-208. ISSN 0098-2997, https://doi.org/10.1016/S0098-2997(02)00017-1 Collins AR. 2005. Assays for oxidative stress and antioxidant status: applications to research into the biological effectiveness of polyphenols. Am J Clin Nutr. 2005 Jan;81(1 Suppl):261S-267S. doi: 10.1093/ajcn/81.1.261S. PMID: 15640489.

Section 6 Adverse effects on sexual function and fertility		
6.1 Human data		
33/18-24, 34/5-13	***Meeker JD et al. Environmental exposure to metals and male reproductive hormones: circulating testosterone is inversely associated with blood molybdenum. Fertil Steril. 2010 Jan;93(1):130-40. doi: 10.1016/j.fertnstert.2008.09.044*** *A significant inverse trend between molybdenum concentrations in blood and testosterone levels.*	In '*Molybdenum is not a risk factor for changes in serum testosterone*' (Klipsch et al 2023) the authors conclude that advanced nonparametric statistical modelling, which expands linear regressions to include more potential variables and common confounders, shows that previously published negative molybdenum-testosterone associations no longer retain statistical relevance. Instead of molybdenum as a causal stressor Klipsch et al 2023 noted several other statistical and scientifically robust explanations for lowered testosterone levels; specifically, body mass index, age-related hormone variability, and underlying global downward trends in testosterone as the appropriate factors responsible for this observation of testosterone decline. IMOA suggests that DECOS revise the text on page 33 to account for the current state of the body of scientific evidence, e.g. adding an introductory clause that reads, 'Conflicting interpretations of the relationship between molybdenum and testosterone have been reported: One paper found …, but a recent (Klipsch et al. 2023) publication indicated an alternate explanation that did not find molybdenum levels to be causal.' Klipsch K, Cox LA, Clark S, Rahim M, Carey S. (2023) Molybdenum is not a risk factor for changes in serum testosterone. Human and Ecological Risk Assessment: An International Journal, 29:5-6, 938-947, DOI: 10.1080/10807039.2023.2218935
6.2 Animal data Note: Mo =molybdenum, SMD = Sodium Molybdate Dihydrate Conversion based on 40% Mo in SMD by molecular weight. Dose conversion factors: EFSA 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579] ttps://doi.org/10.2903/j.efsa.2012.2579		
36/20-32 37/1-8	**Jeter, M.A., Davis, G.K., 1954. The effect of dietary molybdenum upon growth, hemoglobin, reproduction and lactation of rats. J Nutr 54, 215–220. https://doi.org/10.1093/jn/54.2.215**	**Dose levels:0, 5, 20, 80, 140 ppm in the diet. Equivalent to 0, 1.8, 7.2 and 12.6 mg SMD/kg bw/day or 0, 0.72, 2.88 or 5.04 mg Mo/kg bw/day [subchronic rat conversion factor 0.09, EFSA 2012].** *See attached Expert Review.* IMOA concurs with the committee opinion that the number of animals is too low. The group size of 4 to 8/sex has insufficient statistical power [OECD 151, OECD 43] to detect adverse effects on any aspect of fertility or histopathological changes in the absence of any historical control

	The committee considers the number of animals too low to draw such a conclusion upon the oestrus cycle. *Histopathologic examination of the testes of infertile males treated with 80 and 140 ppm molybdenum revealed degeneration of the seminiferous tubules*.	data [EPA 2024]. There is no explanation why the group size is not consistently reported between Tables 1 to 3. In addition, the study fails to provide any details on methodology and very little data to support the conclusions. It was published in 1954 in an era when there were no standard experimental designs for evaluating reproductive toxicity or developmental toxicity in laboratory animals and would not come close to meeting any regulatory guidance, even as a screening study, and therefore should not be regarded as scientifically valid testing. Rats were fed an unconventional, artificial diet made up of only milk powder and sucrose plus only two minerals (iron, manganese) and one vitamin (Vitamin B1). This is unacceptable husbandry, in particular considering the initial age of the test animals; the juvenile and adult animals were not only likely deficient in vitamin status, but in particular lacking zinc as an essential element, a condition known to be related to growth retardation and fertility impairment. Even with the supplementation of copper this diet is not appropriate for studying reproduction as sucrose diets are known to be estrogenic [Sadowska 2022, Thigpen 1987]. The animals were fed diets containing 5 or 20 ppm of copper, which are low and adequate dietary copper levels, respectively. The statement that 75% of the males were sterile at 80 and 140 ppm in the diet fails to note that these results are for rats on the low copper diet, not the adequate copper diet. Only one group was given Molybdenum, at 80 ppm in the diet, and adequate copper. The other groups received low copper and molybdenum at 20, 80 or 140 ppm; there was evidence of copper deficiency (achromotrichia) at the higher dose levels. Histopathology was only performed on 6 infertile male rats and there was no comparison with control males and no evidence whether the same lesions occurred in fertile males. Seminiferous tubule degeneration is common even in fertile control animals. These data cannot therefore be considered reliable. **Klimisch 4.** US EPA; 2024: Guidelines for Reproductive Toxicity Risk Assessment. https://www.epa.gov/risk/guidelines-developmental-toxicity-risk-assessment OECD 2013. Guidance document supporting OECD test Guideline 443 on the Extended One Generation Reproductive Toxicity Test. Series on Testing and Assessment No. 151. ENV/JM/MONO (2013)10 OECD 2008. Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment. OECD Series on Testing and Assessment (No. 43), Organisation for Economic Cooperation and Development, Paris.

		Sadowska J, Dudzińska W, Dziaduch I. 2022.The Effect of Alternating High-Sucrose and Sucrose Free-Diets, and Intermittent One-Day Fasting on the Estrous Cycle and Sex Hormones in Female Rats. Nutrients. 17; 14(20):4350. doi: 10.3390/nu14204350. PMID: 36297033; PMCID: PMC9611605 Thigpen JE, Lebetkin EH, Dawes ML, Richter CB, Crawford D. 1987. The mouse bioassay for the detection of estrogenic activity in rodent diets: III. Stimulation of uterine weight by dextrose, sucrose and cornstarch. Lab Anim Sci 37:606-609
37/9 -35 38/1-2	**Schroeder HA, Mitchener M. 1971. Toxic effects of trace elements on the reproduction of mice and rats. Arch Env Health. 23: 102-106** *The study is poorly reported and no definite conclusion can be drawn based on the data available. There are some indications of an effect on reproduction in the F2 and F3 generation*.	**Dose level: 10 ppm 'molybdate' in the drinking water of mice is equivalent to 1.5 mg/kg bw/day for subchronic exposure [EFSA 2012]. If the test material was SMD, the level of molybdenum would be even lower at 0.6 mg/kg bw/day. Control untreated drinking water.** IMOA concur with the committee opinion that the study is poorly reported. In addition, the study design does not follow any international guidelines and is inadequate for detection of effects on reproduction, owing to the very limited parameters reported. Only 5 pairs of mice were administered molybdenum at a nominal 10 ppm in drinking water but there is no indication that the water was analysed for verification of concentration and the lack of water intake data means actual dose levels cannot be determined. It should be noted that dose levels, when not adjusted during the study can vary substantially, particularly for females in late lactation and for F1 pups [Beekhuijzen et al 2016]. It is unlikely, however, that 1.5 mg/kg bw/day molybdate would produce the deaths observed in the experiment when 40 mg Mo/kg bw/day in the diet and drinking water in a 2-generation rat study was well tolerated [Murray et al 2019]. The diet was non-standard combination of rye flour, dried skimmed milk and corn oil with added vitamins and the water was de-ionised water from a forest spring. The impact of these unconventional nutrition sources over several generations cannot be disregarded [Kennedy & Mitra 1963, Jean-Faucher 1982, Schneider 2004]. The test material is referenced as 'molybdate' so the molybdenum content cannot be confirmed but was likely soluble salt and therefore the dose levels of molybdenum would be lower than stated. Animals were selected prior to treatment from 'divided litters' so males and female pairs may have been siblings at the start of the study and selection of pups for subsequent generations was random so these pairs may also have been related. Pairs were allowed to breed unrestricted for up to 6 months of age. If males were not removed from the cage during littering and lactation, it is possible that maternal care was adversely affected and that cannibalisation of

		dead or abnormal pups accounts for many of the intergroup differences. Animals not selected for further pairings were discarded without examination. The only parameters reported were survival of the parental animals, intervals between litters, failure to breed, age of parental animals at first litter, and litter parameters of litter size, sex ratio, stillborn, number of runts (a subjective assessment only defined as animals with large heads and small bodies), congenital abnormalities and deaths. There is no indication of how frequently the litters were observed and when the first observations were made (i.e. at birth, Day 1 post-partum, later ??). 'Young deaths' are reported but this term is not defined. Similarly, the early deaths (not defined) are reported as a total for the group rather than per litter so the influence of maternal care and litter size cannot be determined and to include the inevitable total litter loss of dead females in these numbers is misleading [OECD 151, OECD 43. As standard deviations are not reported, it is unclear whether differences are due to small differences in every litter or whether specific dams are affected. This is important as large litters tend to be born earlier [Rughe 1968]. The 'litter effect' is not accounted for, as e.g. 'young deaths' are reported for the group, with no range or standard deviation reported [Lazic & Essioux 2013, Gray & Gray 2006, Hotchkiss et al 2008, OECD 151, OECD 43]. The age at first litter is reported for the pair, with no indication of how this single figure for both animals is derived. The average age at littering is 64 to 80 days in the F1 generation, 54 to 89 in the F2 and 54 to 79 in the F3. Since the gestation period of a mouse is 19-20 days, this implies that animals were mated at 5 weeks or earlier in some groups and were therefore likely to be peripubertal and mated at the first oestrus, which may explain some of the variability in the litter data, particularly in the F2 & 3 generations [Evans 1986, Gaytan 2017]. A comparison with the protocols used by NTP for continuous breeding studies in mice and rats [Gulati et al 1991, Chapin & Sloane 1997, Morrisey et al 1989] illustrates the deficiencies of this study design. The above inadequacies in study design clearly indicate that the results cannot be reliably interpreted and are inadequate for any assessment of reproductive function. **Klimisch 3.** Beekhuijzen M, Barentsen H, Marsden E, Zmarowski A, Aujoulat M, Picut C, Sloter E. 2016 Implementing the extended one-generation reproductive toxicity study (EOGRTS): important points to consider. Crit Rev Toxicol.46(4):332-47. doi: 10.3109/10408444.2015.1137863. Kennedy GC, Mitra J. 1963. Body weight and food intake as initiating factors for puberty in the rat. J Physiol. 66(2):408-18. doi: 10.1113/jphysiol.1963.sp007112. PMID: 14031944; PMCID: PMC1359337.

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37/4 - 16	**Fungwe TV et al 1990. The role of dietary molybdenum on estrous activity, fertility, reproduction and molybdenum and copper enzyme activities of female rats, Nutrition Research: 10 (5) 1990: 515-524. ISSN 0271-5317,https://doi.org/10.1016/S0271-5317(05)80061-2.** *At 10 mg/L and higher, oestrus cycle lengths were statistically significantly prolonged compared to control females ($p < 0.05$). The day of oestrus appeared to be extended by 6-12 hours in a majority of the 10 -100 mg molybdenum supplemented animals.*	**Dose levels: 0, 5, 10, 50, 100 mg/L sodium molybdate dihydrate in the drinking water, equivalent to 0, 0.09, 0.45, 0.9, 4.5 and 9.0 mg SMD/kg bw/day or 0, 0.18, 0.36,1.8 and 3.6 mg Mo/kg bw/day [rat subchronic conversion factor 0.09, EFSA 2012]** As noted on Page 39, lines 9 to 23 [Murray et al 2023], the prolonged oestrus cycle reported by Fungwe at 1.5 mg SMD/kg bw/day [~0.36 mg Mo/kg bw/day] was not confirmed by a GLP compliant study using higher dose levels and a similar study design. Murray also notes that amongst other limitations, the Fungwe study evaluated oestrous cycles in only 6 animals per group. This is inadequate statistical power for such a subjective parameter which requires proficiency, training and experience of the personnel reviewing the cytology materials to achieve consistency and avoid prolonged diestrous due to excessive stimulation [OECD 151, OECD 43]. The references used to support the methods for vaginal cytology date from 1964 and 1971 and have been replaced by more detailed methodology [Cooper 1993, Goldman 2007, Cora 2015]. The reporting of the oestrous cycle data only as a mean cycle length for the entire per group does not comply with conventional reporting parameters as it can easily be influenced by the stage of estrous for each animal at the start of the examination and, particularly when numbers examined are so low, an abnormal cycle in a single animal.[Owing to inherent variability, OECD 443 recommends that females are selected for the study on the basis of regular cycling, as per Goldman 2007, and that a minimum of 20 regular cycling females per group is required.] To

		suggest that the oestrus stage had been extended by 6 to 12 hours would require smears to be taken every 6 hours in a 24hr period, which is highly unlikely: More likely is that this is a mean value and it cannot be determined whether this is a moderate effect in all animals or a major difference in an atypical animal. It is notable that ATSDR determined that Fungwe 1990 suffered from “definitely high risk of bias” since the study design or analysis did not account for important confounding and modifying variables. ATSDR also found “probably high risk of bias” due to lack of confidence in the exposure characterization. These study biases further limit the reliability of Fungwe et al. and therefore these data should be considered unsuitable for assessment for classification purposes. **Klimisch 3.** OECD 151. 2013. Guidance document supporting OECD test Guideline 443 on the Extended One Generation Reproductive Toxicity Test. Series on Testing and Assessment No. 151. ENV/JM/MONO (2013)10 OECD 43. 2008. Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment. OECD Series on Testing and Assessment (No. 43), Organisation for Economic Cooperation and Development, Paris. Cooper RL, Goldman JM, Vandenbergh JG. 1993. Monitoring of estrouscyclicity in the laboratory rodent by vaginal lavage. In: Chapin RE,Heindel JJ, editors. Methods in toxicology, Vol. 3: female reproduc-tive toxicology. New York: Academic Press. p 45–56 Goldman, J.M., A.S. Murr, A.R. Buckalew, J.M. Ferrell and R.L. Cooper (2007), “The Rodent Estrous Cycle: Characterization of Vaginal Cytology and its Utility in Toxicological Studies”, Birth Defects Research, Part B, 80 (2), 84-97 Cora MC, Kooistra L, Travlos G. 2015. Vaginal cytology of the laboratory rat and mouse: Review and criteria for the stating of the estrous cycle using stained vaginal smears. Toxicologic Pathology. 4:776-793. Goldman JM, Murr AS, Cooper RL. 2007. The rodent estous cycle: characterization of vaginal cytology and its utility in toxicology studies. Birth Defects Research (B) 80:84-97

38/17-35	**Howell JM, Shunxiang Y, Gawthorne JM. Effect of thiomolybdate and ammonium molybdate in pregnant guinea pigs and their offspring. Res Vet Sci. 1993 Sep;55(2):224-30. doi: 10.1016/0034-5288(93)90085-t.**	**Dose levels: 130.29 µmol Mo/litre as ammonium molybdate, 260.58 µmol Mo/litre as tetrathiomolybdate [No body weight or water intake data reported and no conversion factors available. Ammonium molybdate estimated as 5 mg Mo/kg bw/day based on drinking water consumption by guinea pig of 200 mL/kg/day. 261 µmol/L (*96 µg/µmol) --> 25000 µg/L (*0.2 L/kg/day) = 5 mg/kg/day]. Control untreated drinking water.** Guinea pigs have many disadvantages for reproductive toxicology studies compared to the traditional species, including limited historical control data, variability in pregnancy rates, small and variable litter size, long gestation, relative maturity at birth, and difficulty in dosing and breeding [Rocca 2009]. With such a small group size and without supporting historical control data it is not possible to determine whether the pregnancy rate observed in this study was attributable to treatment when control data from a study with much larger group sizes (24) reports pregnancy rates of 65% [Rocca 2009]. The number of fetuses, resorptions and stillborn pups is reported as a group total, ignoring the litter effect [Lazic & Essioux 2013, Hotchkiss et al 2008, OECD 151, OECD 43). These data are therefore unreliable and not suitable for classification purposes. **Klimisch 3.** Rocca MS, Wehner NG. 2009. The guinea pig as an animal model for developmental and reproductive toxicology studies. Birth Defects Res B Dev Reprod Toxicol.86(2):92-7. doi: 10.1002/bdrb.20188. PMID: 19306306 Lazic SE & Essioux L.2013. Improving basic and translational science by accounting for litter-to litter variation in animal models. BMC Neuroscience 14, 37. 10.1186/1471-2202-14-37 Hotchkiss, A. K., Rider, C. V., Blystone, C. R., Wilson, V. S., Hartig, P. C., Ankley, G. T., Foster, P. M., Gray, C. L., and Gray, L. E. (2008). Fifteen years after "Wingspread"--environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. Toxicol Sci 105(2), 235-259 OECD 2013. Guidance document supporting OECD test Guideline 443 on the Extended One Generation Reproductive Toxicity Test. Series on Testing and Assessment No. 151. ENV/JM/MONO (2013)10 OECD 2008. Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment. OECD Series on Testing and Assessment (No. 43), Organisation for Economic Cooperation and Development, Paris

39/1-22	**ECHA. Registration dossier Disodium molybdate. European Chemicals Agency; 2021.https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/15826** **[A Dose-Range-Finding Reproduction Study of Sodium Molybdate Dihydrate in Rats: CRL Study Number 20062700. Hoberman 2016]**	**Dose levels: 0,3,20,40 mg Mo/kg bw/day in the diet; 3,20,40 mg Mo/kg bw/day in the drinking water, administered as ppm SMD** In this dose-range finding study for the subsequent 2-generation study, males and females were dosed with SMD via the diet and drinking water for 10 weeks prior to pairing until termination at week 14 for males and lactation Day 21 for females. Terminal body weight in males was 11.5 and 11.6% lower than controls in the 40 mg Mo/kg bw/day diet and water exposure groups, respectively. A full array of reproductive parameters was assessed [oestrus evaluation, reproductive capacity, maternal behaviour, natural delivery observations, survival, growth and development of F1 pups, gross necropsy observations, ovarian and uterine examinations, male reproductive assessments including sperm motility, concentration and motility, organ weights, histology and pathological evaluations of kidney, testis, uterus and ovaries]. Pregnancy rate in the 40 m Mo/kg bw/day water exposure group was lower (6/10) than controls but as there was no effect in the dietary exposure group at the same dose level was considered unlikely to be related to the test substance. There were no adverse effects on reproductive parameters including F1 litters to Day 21 post-partum. IMOA notes that, although preliminary, this was a robust study with a group size of 20 per dose level (10 for each route) including full seminology, reproductive organ weights and histopathology of the testis with no effect of treatment a dose levels up to 40 mg Mo/kg bw/day in the diet or drinking water. The results of Pandey & Singh 2002 were not reproduced in this OECD guideline & GLP compliant, higher-powered study of a longer duration (7 days/week), higher dose level and using a well-characterised standard rat model.
39/23-36 40/7-8	**Murray, F.J., Sullivan, F.M., Hubbard, S.A., Hoberman, A.M., Carey, S., 2019. A two-generation reproductive toxicity study of sodium molybdate dihydrate administered in drinking water or diet to Sprague-Dawley rats. Reproductive Toxicology 84, 75–92.** https://doi.org/10.1016/j.reprotox.2018.11.004	**Dose levels: 0, 5, 7, 40 mg Mo/kg bw/day in the drinking water, 0, 40 mg Mo/kg bw/day in the diet. Administered as ppm SMD.** [The achieved dose levels for the high dose dietary and water administrations were similar at 39.7 and 41.3 mg Mo/kg bw/day for P males and 39.0 and 41.3 mg Mo/kg bw/day for F1 males.] IMOA notes that the achieved high dose level for both routes in this study was ~40 mg Mo/kg bw/day [100 mg SMD/kg bw/day], i.e. twice the high dose of 20 mg Mo/kg bw/day [50 mg sodium molybdate dihydrate/kg bw/day] used by Pandey & Singh 2002. The additional high dose group and results from each of two generations greatly increases the statistical power of the study to detect effects [OECD 151, OECD 43]. Exposure was also 7 days per week for a

	The committee considered the applied doses too low for evaluation for adverse effects on fertility.	minimum of 20 weeks in each of 2 generations, whereas in Pandey & Singh the test material was administered only 5 days/week for 60 days, with a maximum of 10 animals examined (actual number not reported). That the results of Pandey & Singh 2002 were not reproduced in this OECD guideline & GLP compliant, higher-powered, longer duration and higher dose level study using a well-characterised standard rat model can only raise uncertainty in the reliability of their data. The dose levels for the 2-generation were selected based on the results of previous sub-chronic and developmental toxicity studies and a range-finding study [Hoberman 2016] in which clear effects were observed in male bodyweights at the top dose level of 40 mg molybdenum/kg bw/day by both routes). In the 90-day study a high dose of 60 mg Mo/kg bw/day produced a 15% reduction in terminal body weight in males, which only reduced to 9% lower than controls after the 60 day recovery period. This dose level was therefore considered too high for the duration of dosing required for the 2-generation study and for pregnant females. Higher dose levels have been investigated over shorter dosing periods but were not tolerated or the response to treatment would be considered excessive for the much longer duration of a 2-generation study (~ 20 weeks compared to~2 weeks dosing): In a preliminary gavage study in non-pregnant female rats 4 animals treated at 400 mg Mo/kg bw/day were found dead on Day 6 and the remaining 2 animals were euthanized. At 240 mg Mo/kg bw/day all animals were terminated after 7 days of dosing due to adverse clinical observations and reductions of 25% in body weight and 64% in food consumption. Females administered 120 mg Mo/kg bw/day survived to scheduled euthanasia on Day 15 but adverse clinical observations, and reductions of 21% in body weight and 40% in food consumption were observed. In a preliminary dietary study in pregnant female rats terminal body weights at gestation day 21 were 13 and 23% lower than controls at 120 and 160 mg Mo/kg bw/day and food intake was reduced by 19 & 39% over the treatment period (gestation day 6 to 21) respectively. In the subsequent prenatal developmental toxicity study, Aveyard 2023 [page 167], lines reports terminal body weights in pregnant animals administered treated diet from gestation day 6 to 21 as 11.2% and 20.7% lower than the control group value for females treated at 80 and 120 mg Mo/kg bw/day, respectively. Food intake was reduced by 11 and 26%, respectively. For all the above detailed reasons supporting appropriate dose range selection, the applied doses cannot be considered inadequate for evaluation for adverse effects on fertility.

		OECD 2013; Guidance document supporting OECD test Guideline 443 on the Extended One Generation Reproductive Toxicity Test. Series on Testing and Assessment No. 151. ENV/JM/MONO (2013)10 OECD 2008. Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment. OECD Series on Testing and Assessment (No. 43), Organisation for Economic Cooperation and Development, Paris Aveyard L, Murray FJ, Hubbard SA, Hoberman AM, Allen BC and Carey S. 2023. OECD 414 supplementary prenatal developmental toxicity study of sodium molybdate dihydrate in the rat and benchmark dose evaluation. Reproductive Toxicology; 120: 108443.
40/22-23	**Murray FJ, Aveyard L, Hubbard SA, Hoberman AM and Carey S. Sodium molybdate dihydrate does not exhibit developmental or reproductive toxicity in Sprague-Dawley rats maintained on a marginal copper diet. Reproductive Toxicology 2023; 120: 108442.** *The prolonged oestrus cycle that Fungwe et al found at 1.5 mg molybdenum /kg bw /day was not confirmed by Murray et al. (2023).*	**Dose levels: 0, 20, or 40 mg Mo/kg bw/day (as SMD) in the drinking water at 0, 169–235 or 337–432 ppm.** IMOA notes that this study in female Sprague-Dawley rats maintained on a semi-purified diet with a marginal copper level was designed to reproduce that of Fungwe 1990 at dose levels which exceeded those reported by Fungwe i.e. 0, 5, 50 or 100 mg/L [estimated by ATSDR as 0.76 to 15 mg Mo/kg bw/day, or 0, 0.45, 4.5 or 9 mg Mo/kg bw/day using the EFSA conversion factor of 0.09, EFSA 2012]. Not only was the prolonged oestrous cycle not confirmed but differences in litter size, the incidence of resorptions, fetal body weight or the length of the oestrous cycle reported by Fungwe could not be replicated. There were no test material-related effects at 20 or 40 mg Mo/kg bw/day on all assessments of female fertility i.e. oestrous cycling, reproductive performance, maternal macroscopic pathology, ovarian or uterine parameters, litter size, resorptions, fetal sex ratio, fetal weight, or external fetal malformations or variations. The author notes that it is clear that a marginal Cu diet is not the explanation for inconsistencies between the results of Fungwe 1990 and the guideline-compliant toxicity studies of molybdenum and that increasing doses (and serum levels) of molybdenum are not associated with copper depletion in serum whether the rats are fed a marginal Cu AIN-93 G diet (6.2 ppm Cu, as per this study) or standard rat chow with an adequate Cu level (12–13 ppm Cu) as per the OECD guideline-compliant toxicity studies [Murray et al 2014, 2019; Aveyard 2023, pages 162,164 and 187]
41/3-5 41/9-10	**NTP, 1997. NTP Toxicology and Carcinogenesis Studies of Molybdenum**	**Dose levels: 0, 3, 10, 30 and 100 mg MoO^3/m^3 5 days/week, 6.5h/day** *See attached Expert Review.*

	Trioxide (CAS No. 1313-27-5) in F344 Rats and B6C3F1 Mice (Inhalation Studies). Natl Toxicol Program Tech Rep Ser 462, 1–269. *No statistically significant effects were observed on sperm count, and on the concentration and motility of epididymal spermatozoa in any of the treatment groups.* *The NTP did not specifically examine sperm pathology.*	This publication reports a programme of inhalation studies with MoO^3 including 13 and 104 week studies in the F344/N rat and B6C3F1 mouse in which organ weight and full histopathology of the reproductive organs was included and, in the 13 week rat study, sperm count and motility. In the 104 week Rat NTP study blood levels of Mo were: 0.22, 0.80, 1.77 and 6.04 μg/g for 3, 10, 30 and 100 mg MoO^3/m^3, respectively, for males. Exposure levels (as blood levels) for males at the high dose in this inhalation study can therefore be compared to week 12 of the 90-day study where they are higher than the 2930 ng/mL (2.93 μg/g) reported for 17 mg Mo/kg bw/day and slightly lower than the 9903 ng/mL (9.9 μg/g) reported for 60 mg Mo/kg bw/day. In mice, as might be expected from the lower lung capacity than rats, exposure levels were lower, with the high dose level of 100 mg MoO^3/m^3 providing results similar to the 30 mg MoO^3/m^3 level in rats. The lack of any adverse effect in the reproductive organs in both species or on sperm parameters in the rat in these GLP compliant NTP-guideline studies at exposure levels within the range of the dietary 90-day and 2-generation studies is further support for no adverse effect on male fertility in GLP compliant, reliable studies.
41/11-33	**Pandey R, Singh SP. Effects of molybdenum on fertility of male rats. Biometals. 2002 Mar;15(1):65-72. doi: 10.1023/a:1013193013142.** *At 50 mg/kg bw, testis, epididymis, seminal vesicles, and prostate gland weights (absolute and/or relative weights) were statistically significantly decreased, and an accumulation of molybdenum was seen in these organs. At 30 mg/kg bw, epididymis weight, absolute weight of seminal vesicles, and relative weight of the prostate gland were statistically significantly decreased. At both concentrations, degeneration of the seminiferous tubules in the testis was*	**Dose levels: 0, 10, 30, 50 mg SMD/kg bw/day; 0, 4, 12, 20 mg Mo/kg bw/day [reported by ATSDR as 0, 4.7, 14, 24 mg Mo/kg bw/day]** *See attached Expert Review.* IMOA notes that the text referenced dose levels are for SMD. Druckery rats are an inbred strain, specific to ITRC Lucknow and not commonly used in regulatory toxicology studies. The lack of historical data is a major issue for interpretation of results as the reproductive performance of this strain is unreported. It is unclear how many animals were used for the various endpoints measured. Although 10/group were dosed, the tables report that the number of animals for each endpoint (molybdenum analysis, testicular enzymes, histopathology) was “the requisite number of animals in each group”, but so few animals means that cannot be the case. Testes were fixed in 10% NBF and have major fixation artifacts precluding assessment of any changes. Histopathology is poorly described. The single picture provided is badly out of focus and it is impossible to determine whether there is any cell degeneration. There is obvious increase in the interstitial space, but this is a common fixation artifact seen in tubules in the

	observed. Epididymal sperm motility and total sperm count (per epididymis) were reduced in the two highest dose groups (although the authors don't account the total sperm count in the highest dose group as significantly lower as compared to the control group).	centre of the testis due to poor penetration of the fixative. The incidence or severity of changes and number of animals examined is not reported. This is essential since untreated rats can have incidental testicular findings in the testes. To conclude a treatment related effect, the incidence and severity of any changes and any dose response must be evaluated. The text states that the 4 mg Mo/kg bw/d testes were almost normal but does not provide any indication of what, if any, changes there were and no incidences or severities of any findings are provided for the mid and high dose groups. Degeneration of interstitial cells would be a very rare and unlikely finding, and it would be expected to lead to a decrease in testosterone production, which would be reflected by atrophy of the seminal vesicles and prostate. There is a small decrease in absolute and/or relative seminal vesicle and prostate weight (not commented on by the authors), which could suggest that the high dose animals were subject to stress or body weight loss. However, although 'Sluggishness' was reported in the high dose group, as no body weight or food intake data are provided it is not possible to assess the condition of the animals. Sperm motility assessment was carried out using crude manual techniques inadequately described (1936 paper referenced). Sperm motility is a variable endpoint that requires carefully controlled methodology and is generally performed using computer assisted sperm analysis (CASA) with adequate Historical Control Data (HCD). Sperm count was measured using caudal sperm and should be reported as count /g of cauda. Instead it is presented per epididymis, which fails to take into account the variable weight of tissue sampled. The decrease in some testicular enzymes (sorbitol dehydrogenase and gamma glutamyl transpeptidase) and the increase in lactate dehydrogenase are meaningless in the absence of any detailed histopathological changes. Although the overall design of this study is adequate, the quality of the data generated is poor and unreliable. The overall conclusions regarding the effects of molybdenum on the testes and epididymis relies on the histopathology and sperm analysis, since these are the most sensitive endpoints for detecting testicular toxicity. However, the fixation of the testes is inadequate and the reporting and description of any findings in the testes is poor and incomplete. The methodology used to measure and report sperm parameters is also inadequate. In the second part of the experiment, a group of 20 male rats of proven fertility treated with 30 mg SMD/kg bw 5 days per week for 60 days, and 20 control distilled water treated rats were mated with untreated 'proven' females on a 1 male:2 females basis for 1 or 2 weeks and the mated females examined on Day 20 of gestation. 'Proven' is not defined but in another publication from the same institute is defined as 'one previous pregnancy'. Use of proven

		females rather than the virgin females required by regulatory guidance (OECD 422) can affect both mating behaviours [Taylor 1989] but also reproductive parameters [Stouffer 2006]. The reference used for vaginal smears taken to confirm pregnancy [Dunnick 1984] is an NTP study of DMMP which does not describe the method, just the 1:2 male to female mating ratio and that daily vaginal smears were taken during the mating period. The paper does not provide the detailed method necessary to support an accurate assessment of mating at the correct stage of the cycle [Young et al 1941.Erbeck et al 1995, Voipio & Nevalainen 1998, Cooper at al 1993, Cooper & Goldman 1995] and this lack of experience alone could affect the reported pregnancy rates of 60% in the treated group and 80% in the controls. The number of females from which this percentage was calculated is not reported, however, and results for Day 20 of gestation are shown in Table 6 for only 10 per group rather than the 40 implied by 1:2 mating of 20 males. A footnote states "*No such results were observed in remaining groups of animal*" but no further groups are described in the method section and it is uncertain whether this means no differences from control occurred or whether the remaining males were not examined. Whether the difference in pregnancy rate is significant would depend on the number examined (statistical power) and historical background data (since this strain has little published data) but such a variation in pregnancy rate is not uncommon in studies with a small group size. Similarly the results in Table 6 cannot be interpreted without historical control data as the group size is too small for the natural variation observed in these parameters. It is unclear whether treatment of males continued after mating. If not, this would allow up to two weeks for the males to recover, introducing further uncertainty into results as the time to mating in the two groups are not reported. No further conclusions can be drawn from this part of the study and the conclusions of the authors about "male-mediated developmental toxicity' are unsupported. Examination of other references used to support the research further suggests inexperience: for example, two references are quoted in the discussion as reporting effects on testis weight and histopathology from sodium molybdate [Davis 1967 and Hoey 1966] but neither of these publications mentions molybdate. Davis investigated cadmium chloride and the antispermatogenic drugs triethylmelamine and N,N' bis[dichloroacetyl)-l,8-octamethylenediamine and Hoey used copper, tin, cobalt, nickel and silver. It is notable that EFSA, in their update of the risk assessment of nickel [Grasl-Kraupp 2020] reported 'Limitations in these studies preclude their use for the establishment of a reference

		point' for publications by this group on the male reproductive effects of nickel [Pandey 1999, Pandey 2000]. **Klimisch 3.** Dunnick JK, Gupta BN, Harris MW, Lamb JC. 1984. Reproductive toxicity of dimethyl methyl phosphonate (DMMP) in the male Fischer 344 rat. Toxicol Appl Pharmacol. 15;72(3):379-87. doi: 10.1016/0041-008x(84)90114-5 Taylor GT, Bardgett M, Griffin M, Weiss J. 1989. Reproductive behavior and physiology of nulliparous female rats after sexual experience. Behavioural Processes. 19 (1-3): 31-46 Stouffer RL. 2006. Structure, function, and regulation of the corpus luteum. In: Knobil and Neill's Physiology of Reproduction, 3rd ed. JD Neill (ed). Elsevier Academic Press, San Diego. 475–526 Young WC, Boling JL, Blandau RJ. 1941. The vaginal smear picture, sexual receptivity and time of ovulation in the albino rat. Anatomical Record: Vol. 80, 37-45 Erbeck DH, Kruckenberg SM. 1995. Vaginal (copulation) plugs in laboratory rodents: a review. Contemp Top Lab Anim Sci. 34(1):71-4. PMID: 16457583 Voipio HM & Nevalainen T. 1998. Improved method for vaginal plug detection in rats. Scandinavian Journal of Laboratory Animal Science, 25(1) https://doi.org/10.23675/sjlas.v25i1.818 Cooper RL, Goldman JM, Vandenbergh JG. 1993. Monitoring the estrous cycle in the laboratory rodent by vaginal lavage. In: Methods in Toxicology. Volume 3B. Female Reproductive Toxicology. Heindel JJ, Chapin RE, eds. Academic Press Inc, San Diego, CA. Cooper RL, and Goldman JM. 1999. Vaginal cytology. In: An Evaluation and Interpretation of Reproductive Endpoints for Human Health Risk Assessment. Daston G, and Kimmel C, eds. pp. 42–56, ILSI Press, Washington, DC. Davis JT, Coniglio JG. 1967. The effect of cryptorchidism. Cadmium and antispermatogenic drugs on fatty acid composition of rat testis. J Reprod Fert 14, 407–413

		Hoey MJ. 1966. The effects of metallic salts on the histology and functioning of the rat testis. J Reprod Fertil. 12(3):461-72. doi: 10.1530/jrf.0.0120461 Grasl-Kraupp B et al. 2020. Update of the risk assessment of nickel in food and drinking water. EFSA J. 5;18(11):e06268. doi: 10.2903/j.efsa.2020.6268 Francis EZ. 1994. Testing of Environmental Agents for Developmental Toxicity. Ch 16. In: Kimmel CA, Buelke-Sam J. Developmental Toxicology. 2nd ed. Raven Press; 1994 US EPA; 2024: Guidelines for Reproductive Toxicity Risk Assessment https://www.epa.gov/risk/guidelines-developmental-toxicity-risk-assessment OECD 2013; Guidance document supporting OECD test Guideline 443 on the Extended One Generation Reproductive Toxicity Test. Series on Testing and Assessment No. 151. ENV/JM/MONO (2013)10 Pandey R, Kumar R, Singh SP, Saxena DK, Srivastava SP. 1999. Male reproductive effect of nickel sulphate in mice. Biometals. 12(4):339-46. doi: 10.1023/a:1009291816033. Pandey R, Srivastava S. 2000. Spermatotoxic Effects of Nickel in Mice. Bull. Environ. Contam. Toxicol. 64, 161–167. https://doi.org/10.1007/s001289910025
41/34-35 42/1-9 Page 115	**IMOA. 28-day oral gavage and dietary administration dose range-finder study in rats. Test substance: sodium molybdate dihydrate. International Molybdenum Association, London, UK: conducted by HLS New Jersey, 2011**. *DECOS comment that the 28 day exposure period may be too short to evaluate testicular toxicity.*	**Dose levels: 0, 4, 20, 2x10 mg Mo/kg bw/day gavage, 0, 4, 20 mg Mo/kg bw/day in the diet. Administered as 0, 10, 50, 2 x 25 mg SMD/kg bw/day gavage or 0, 125, 625 ppm SMD in the diet.** IMOA notes that the dose levels and duration of exposure in this preliminary study exceed that of Zhai 2013 and the duration is similar to that of Khorami 2020, with much higher dose levels. The duration of exposure is conventional for dose range-finding studies for subsequent 90-day studies and is not intended as a full assessment of spermatogenesis. Nevertheless, there were no differences from control in absolute or relative testes or epididymides weights. Histopathology of the testes and epididymides revealed minimal tubular degeneration/atrophy of the tubules in all groups, with the highest incidence in the gavage control group: this finding was considered an incidental, background finding (supported by historical control data).

42/10-32	**Murray, F.J., Sullivan, F.M., Tiwary, A.K., Carey, S., 2014. 90-Day subchronic toxicity study of sodium molybdate dihydrate in rats. Regulatory Toxicology and Pharmacology 70, 579–588.** https://doi.org/10.1016/j.yrtph.2013.09.003 Publication of: International Molybdenum Association. Sodium molybdate dihydrate: a 90-day oral dietary study in rats (GLP). IMOA, London, UK: prepared by Huntingdon Life Sciences, USA, 2011	**Dose levels: 0, 5, 17, 60 mg Mo/kg bw/day. Administered as 0, 150, 500 & 1750 ppm SMD in the diet.** Chapin et al 1998 compared reproductive system necropsy data from general toxicity studies, including sperm motility and vaginal cytology evaluations, and concluded that data from 90-day studies can provide a valuable indication of the likely reproductive toxicity of the compound under study. As the Murray 2014 study included these parameters, it can be considered reliable weight of evidence for no adverse effect of molybdenum on male reproductive parameters, The findings of Pandey & Singh 2002, from a 5/day week 60 day study at lower dose levels could not be reproduced. Chapin RE, Sloane RA, Haseman JK. 1998. Reproductive endpoints in general toxicity studies: are they predictive? Reprod Toxicol. 12(4):489-94. doi: 10.1016/s0890-6238(98)00026-4.
43/1-10	**Zhai, X.-W., Zhang, Y.-L., Qi, Q., Bai, Y., Chen, X.-L., Jin, L.-J., Ma, X.-G., Shu, R.-Z., Yang, Z.-J., Liu, F.-J., 2013. Effects of molybdenum on sperm quality and testis oxidative stress. Syst Biol Reprod Med 59, 251–255.** https://doi.org/10.3109/19396368.2013.791347 *The results showed that the sperm parameters, including the epididymis index, sperm motility, sperm count, and morphology, increased by a moderate dose of molybdenum (5 mg/kg bw/day), but were negatively affected at high doses (≥ 20 mg/kg bw/day). Results for the abnormality rate ((no. sperm with abnormal morphology/ no. total spermatozoa) × 100) were consistent with those findings, showing a decrease of abnormality at 5 mg/kg bw/day and an increase at 20 and 40 mg/kg bw/day. In*	**Dose levels: 0, 12.5, 25, 50, 100, 200 mg SMD/L in drinking water is equivalent to 0, 2.25, 4.5, 9.0, 18.0, 36 mg SMD/kg bw/d or 0, 0.9, 1.8, 3.6, 7.2, 14.4 mg molybdenum/kg bw/day [conversion factor 0.18 for mouse subacute, EFSA 2012]** *See attached Expert Review.* A major limitation of this study is the absence of any information on molybdenum intake by the mice and the sexual immaturity of the animals. Body weight gain, water intake or clinical condition of the mice are not reported so the condition the higher dose animals is unknown. Testis weight was not measured and no histopathology was performed. If sperm parameters were affected by treatment then the duration of dosing (14 days) would infer such changes were due to effects occurring in the epididymis and not the testes, so the conclusions of the study are not supported by the study design. The age of the mice (3-4 weeks at the start of dosing) would result in a lot of variability in sperm parameters because they would be in peripubertal status with some animals reaching sexual maturity ahead of others. This may explain the apparent improvement in sperm parameters in some groups compared with controls. It may also explain the extremely high rate of abnormal sperm in the control group (>28%) whereas the expected rate for ICR mice is 6.8% (https://www.criver.com/sites/default/files/noindex/historical-control-data/hcd-pa-mice.pdf). The description of methodology for measuring sperm parameters and testicular enzymes is rudimentary and lacking any detail. The study design is considered flawed and the results unreliable.

	addition, the changes of sperm parameters were accompanied with changes of the superoxide dismutase (SOD) activities, the glutathione peroxidase (GPx) activities, and the malondialdehyde (MDA) levels in testes.	The methods for measurement of SOD, GPx and MDA report use of commercial kits but do not report that they had been validated for the species and/or under the conditions of the laboratory. Without such assurance, or any historical control data, the reported results cannot be considered reliable [Zhou 2006, Griffiths 2002, Collins 2005.Tsikas 2017, Michel 2008]. **Klimisch 3.** Zhou JY, Prognon P. 2006. Raw material enzymatic activity determination: A specific case for validation and comparison of analytical methods—The example of superoxide dismutase (SOD), Journal of Pharmaceutical and Biomedical Analysis. 40 (5):1143-1148. ISSN 0731-7085, https://doi.org/10.1016/j.jpba.2005.09.022. Griffiths HR, Møller L, Bartosz G, Bast A, et al. 2002. Biomarkers. Molecular Aspects of Medicine Volume 23, Issues 1–3, Pages 101-208. ISSN 0098-2997, https://doi.org/10.1016/S0098-2997(02)00017-1 Collins AR. 2005. Assays for oxidative stress and antioxidant status: applications to research into the biological effectiveness of polyphenols. Am J Clin Nutr. 2005 Jan;81(1 Suppl):261S-267S. doi: 10.1093/ajcn/81.1.261S. PMID: 15640489 Tikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem. 2017 May 1;524:13-30. doi: 10.1016/j.ab.2016.10.021. Epub 2016 Oct 24. PMID: 27789233 Michel F, Bonnefont-Rousselot D, Mas E, Drai J, Thérond P. Biomarqueurs de la peroxydation lipidique: aspects analytiques [Biomarkers of lipid peroxidation: analytical aspects]. Ann Biol Clin (Paris). 2008 Nov-Dec;66(6):605-20. French. doi: 10.1684/abc.2008.0283. PMID: 19091659
43/11-23	**Zhang YL, Liu FJ, Chen XL, Zhang ZQ, Shu RZ, Yu XL, Zhai XW, Jin LJ, Ma XG, Qi Q, Liu ZJ. Dual effects of molybdenum on mouse oocyte quality and ovarian oxidative stress. Syst Biol Reprod Med. 2013 Dec;59(6):312-8. doi: 10.3109/19396368.2013.826296.**	**Dose levels:0, 5,10,20,40 mg SMD/L drinking water is equivalent to 0, 0.9, 1.8,3.6,7.2 mg SMD/kg bw/d or 0, 0.36, 0.72, 1.44, 2.88 mg Mo/kg bw/day [conversion factor 0.18 for mouse subacute, EFSA 2012]** *See attached Expert Review* The female mice were aged 4 to 6 weeks at the start of treatment, and therefore 6 to 8 weeks at termination when the ovaries were sampled. As the average age of vaginal opening in the mouse is ~4 weeks [Historical control data for Crl:CD (ICR) Mice,

	Compared to the control group, metaphase II oocyte morphology, ovary index (ovary weight/total body weight), and ovulation improved within the 1 mg/kg bw/day group, but were negatively affected by sodium molybdate dihydrate at 8 mg/kg bw/day. These alterations were accompanied by changes in superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) levels in ovaries. Morphologically abnormal ovarian mitochondria were observed at ≥ 4 mg/kg bw/day.	https://www.criver.com/products-services/safety-assessment/toxicology-services/developmental-and-reproductive-toxicology-dart/historical-control-data?region=3696], the (unknown) mix of peripubertal and sexually mature females within each group is a major confounding factor for assessment of ovarian function. The ages quoted are also inconsistent with the claim that females were 'of proven fertility', which implies previous mating history (which would in itself be a confounding factor). The females were also superovulated by IP injection of 10 IU PMSG (pregnant mare serum gonadotrophin) followed by 10 IU of hCG (human chorionic gonadotrophin), a technique designed to induce ovarian follicular development and oocyte maturation, and therefore inappropriate for assessing oocyte quality from unstimulated animals. The number of oocytes/embryos collected from a superovulated female mouse may be influenced by several factors including the age/weight of the mouse, the dosage given and the skill of the person administering the injections. The hormone dosage is extremely sensitive in increasing or decreasing ovulation and the number of oocytes/embryos, and relies on accurate administration of the exact dose in all animals. The hormone dosage is usually between 2.5 IU and 5.0 IU.[Luo et al 2011, Shindo et al 2022.] The methodology section is very brief and lacking detail. It suggests 25 mice/group were treated with molybdenum but does not state whether all were examined for Transmission Electron Microscopy (TEM) and this is very unlikely given the time consuming nature of TEM preparation and evaluation. The incidence of mitochondrial vacuolation is not provided, rather the authors provide inadequate poor photomicrographs. In the rodent ovary, oocytes are continuously developing but the majority (>90%) will undergo atresia. The different stages of development of the oocyte have been divided into 3 different stages (small medium and large) and these oocytes are contained within follicles that have been divided into 10 different stages. The studies do not take account that the ultrastructure of the oocyte and of the granulosa cells will be changing continuously through this development and maybe undergoing growth or death. Mitochondrial vacuolation is a normal feature of atresia. Oocyte morphology is not a recognised technique for detecting ovarian toxicity. As with TEM, the number of mice sampled was not reported and it is not clear how the data were analysed - on a per animal basis or whether all oocytes were pooled for analysis. The stage of the estrous cycle was not accounted for and would very likely affect the hyperaemia of the ovary (for which no incidence was reported). Similarly, the time elapsed after the hormone injection was not reported. The recognised way of evaluating ovarian toxicity that is recommended by the regulatory guidance is a quantitative analysis of primordial and small growing follicles [OECD TG 443,

		OECD GD151]. The Murray 2019 2-generation study included this examination and no changes were observed in either generation. The results in this 2-generation study can also be integrated with all the other important endpoints for evaluating female reproduction (estrous cyclicity, fertility parameters, etc). The data from this GLP study using validated, quantitative (and very time consuming) techniques are therefore more robust and the duration of exposure of the P and F1 animals much longer. The methods for measurement of SOD, GPx and MDA report use of commercial kits but do not report that they had been validated for the species and/or under the conditions of the laboratory. Without such assurance, the reported results cannot be considered reliable [Zhou 2006, Tsikas 2017, Michel 2008] The above deficiencies therefore indicate that then study outcomes cannot be considered reliable. **Klimisch 3**. Luo C, Zuñiga J, Edison E, Palla S, Dong W, Parker-Thornburg J. 2011. Superovulation strategies for 6 commonly used mouse strains. J Am Assoc Lab Anim Sci. 50(4):471-8. PMID: 21838974 Shindo M, Miyado K, Kang W, Fukami M, Miyado M. 2022. Efficient Superovulation and Egg Collection from Mice. Bio Protoc. 5;12(11):e4439. doi: 10.21769/BioProtoc.4439 OECD (2013): Guidance Document supporting TG 443: Extended One Generation Reproductive Toxicity Study, Series on Testing and Assessment, No. 151, OECD, Paris ENV/JM/MONO (2013)10 OECD (2018), Test No. 443: Extended One-Generation Reproductive Toxicity Study, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264185371-en. Zhou JY, Prognon P. 2006. Raw material enzymatic activity determination: A specific case for validation and comparison of analytical methods—The example of superoxide dismutase (SOD), Journal of Pharmaceutical and Biomedical Analysis. 40 (5):1143-1148. ISSN 0731-7085, https://doi.org/10.1016/j.jpba.2005.09.022

		Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem. 2017 May 1;524:13-30. doi: 10.1016/j.ab.2016.10.021. Epub 2016 Oct 24. PMID: 27789233. Michel F, Bonnefont-Rousselot D, Mas E, Drai J, Thérond P. Biomarqueurs de la peroxydation lipidique: aspects analytiques [Biomarkers of lipid peroxidation: analytical aspects]. Ann Biol Clin (Paris). 2008 Nov-Dec;66(6):605-20. French. doi: 10.1684/abc.2008.0283. PMID: 19091659
43/34-35 44/1-20	**Wang, H, Zhou B, Zhang S, Guo H, Zhang J, Zhao J, Tian E, 2016. Reproductive toxicity in male mice after exposure to high molybdenum and low copper concentrations. Toxicol Ind Health 32, 1598–1606.** https://doi.org/10.1177/0748233715569269 *The authors concluded that administration of molybdenum decreased sperm density and increased the rate of teratosperm occurrence. Histopathological examination of testicular tissue showed slight histological alterations in animals treated with molybdenum. Degenerated and atrophic germinal cells were detected in the atrophic lumina of the tubules in the high molybdenum group. The testicular tissues and cells were more seriously damaged when molybdenum was administered with cupper deficiency. "Spermatogenic cells" also showed morphological changes in the high molybdenum group, including reduced amounts of chromatin, cellular nuclear volume loss endoplasmic reticulum dilation, and nuclear membranebreakage or disappearance. The mitochondria of spermatogenic cells and*	**Dose levels: 400 mg/L drinking water, equivalent to 60 mg/kg bw/day of 'molybdenum' – not further described [mouse subchronic conversion factor of 0.15, EFSA 2012]. Control untreated water.** *See attached Expert Review.* The chemicals selected and method to add Cu and Mo to the drinking water are not described. Molybdenum was administered in drinking water but water intake was not measured so the dose level can only be estimated. It is likely that a soluble salt was dissolved in the drinking water, and therefore the above dose level is overstated – eg if the material were SMD, then the dose level would be equivalent to 24 mg/kg bw/day Information on group size is conflicting: On page 2, it is reported that: "80 mice were weighed and divided into 4 groups of 20." and "On the 50th and 100th days of treatment, 10 mice were randomly selected from each group …". whereas the tables report results for only 6 male mice per group, not 10, without explanation. A group size of 6 is totally inadequate for this type of study to obtain statistically meaningful results. Histopathology was performed on paraformaldehyde fixed tissue, which has provided very poor fixation. Despite the authors claiming there is major disruption of spermatogenesis in the treated testes, all of the testes appear to suffer from major fixation artifact to the same degree, especially in the peripherally located tubules. There are no organ weights and there is negligible detail for the methodology used for sperm assessment, histopathology and TEM. What little information there is demonstrates that the methodology is very basic and uses inappropriate sampling techniques and fixatives. The description and photomicrographs of testicular histopathology and ultrastructure indicate that the authors have very limited knowledge of the normal features of the seminiferous tubules and their cell types. The methods for measurement of SOD, GPx and MDA report use of commercial kits but do not report that they had been validated for the species and/or under the conditions of the laboratory. Without such assurance, the reported results cannot be considered reliable. These data are therefore considered unreliable. **Klimisch 3.**

	sperms showed extensive vacuolization, were swollen and were less dense than those of the controls. A significant increase in malondialdehyde content and a decrease in superoxide dismutase and total antioxidant capacity contents in testicular tissue was observed in the high molybdenum group.	Zhou JY, Prognon P. 2006. Raw material enzymatic activity determination: A specific case for validation and comparison of analytical methods—The example of superoxide dismutase (SOD), Journal of Pharmaceutical and Biomedical Analysis. 40 (5):1143-1148. ISSN 0731-7085, https://doi.org/10.1016/j.jpba.2005.09.022. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem. 2017 May 1;524:13-30. doi: 10.1016/j.ab.2016.10.021. Epub 2016 Oct 24. PMID: 27789233. Michel F, Bonnefont-Rousselot D, Mas E, Drai J, Thérond P. Biomarqueurs de la peroxydation lipidique: aspects analytiques [Biomarkers of lipid peroxidation: analytical aspects]. Ann Biol Clin (Paris). 2008 Nov-Dec;66(6):605-20. French. doi: 10.1684/abc.2008.0283. PMID: 19091659.
44/21-34	**Khorami, H., Eidi, A., Mortazavi, P., Modaresi, M., 2020. Effect of sodium molybdate on cadmium-related testicular damage in adult male Wistar rats. Journal of Trace Elements in Medicine and Biology 62, 126621.** https://doi.org/10.1016/j.jtemb.2020.126621 *No effects on sperm count, sperm viability, sperm morphology, sperm membrane integrity or sperm motility were observed upon treatment with sodium molybdate. Additionally, no effects of sodium molybdate were seen on oxidative stress parameters. Histopathology of seminiferous tubules in the animals treated with sodium molybdate showed normal spermatocytes, spermatids and spermatozoa. The level of aquaporin 9 protein expression in the testicular tissues, was not affected by sodium molybdate. The committee considered*	**Dose level: 0, 0.05, 0.1, 0.2, 0.4 mg SMD /kg bw/day, 0.02, 0.04, 0.08, 0.16 mg Mo/kg bw/day by gavage.** *See attached Expert Review.* In contrast to other publications this study appears to be well conducted, although not GLP compliant. CASA was used to assess sperm motility but the methodology may not be validated or as used in other laboratories eg entire epididymis appears to have been used to obtain sperm for analysis whereas the cauda and vas deferens are routinely used in regulatory studies. Bouin's fixative was used to fix the testes. Although the photomicrographs of testicular histopathology lack resolution, they are adequate to demonstrate the normal structure of the sodium molybdate treated testes and the significant changes in the cadmium chloride-treated testes. Histopathological changes have been assessed using a semi-quantitative scoring system (Johnson's score) and the data tabulated. The study demonstrates that gavage dosing with sodium molybdate dihydrate at dose levels of up to 0.16 mg molybdenum/kg/kg bw/d for 30 days has no effects on sperm parameters, histopathology or lipid peroxidation/oxidative enzymes of the testis and conversely, improves these parameters in rats dosed with the potent testicular toxicant CdCl2. **Klimisch 2.**

	the doses as applied as too low to observe relevant effects.	
6.3 Data Evaluation		
44/1-11	*For animal studies with molybdates, there are indications that the substances may affect the male reproduction system, i.e. reduced sperm count and quality. Although this is not translated into a clear effect on reproduction performance in the most recent studies, the committee is of the opinion that these reproduction studies were performed at doses that were too low to introduce parental toxicity. The effect of molybdenum on a prolonged oestrus cycle did not provide sufficient evidence according to the committee.* *Overall, the animal studies are indicative of effects on male rats and mice, but no conclusion can be drawn on whether exposure functionally affected fertility*	IMOA commissioned a review of adverse male reproductive effects by a renowned expert in male reproductive histopathology, Dr Dianne Creasy, which concludes that the data from most non-GLP, non-Guideline studies (with the exception of Khorami 2022) are unreliable and unsuitable for classification purposes and that the toxicity studies that have been performed according to OECD/NTP guidelines in GLP compliant laboratories (NTP 1997, Murray 2014, 2019) stand out as providing the most reliable and statistically robust data on which to base any hazard identification of molybdenum compounds. The dose levels used in the IMOA 2-generation and associated range-finding studies and the 90-day study are higher, in terms of molybdenum content, than those of Pandey & Singh on which the previous classification was based. That these studies, and the NTP rat and mouse 13 week repeat dose and 104 week carcinogenicity studies at similar blood exposure levels did not discover any effects of treatment on male reproductive organs and the 2-generation and range-finding studies did not reveal any functional effect on fertility and reproductive outcomes strongly refutes any effect observed in less reliable experimental studies at lower dose levels of molybdenum.
6.4 Conclusion		
48/22-26	*Based on animal studies that are indicative of reproductive effects in males, the committee is of the opinion that molybdenum and selected inorganic molybdenum compounds need to be classified as Category 2 (Suspected human reproductive toxicant (H361f)).*	The Expert Review of reported adverse male reproductive effects by a renowned expert in male reproductive histopathology, Dr Dianne Creasy, concludes that the data on sperm count and quality from most non-GLP, non-Guideline studies (with the exception of Khorami 2022) in rats and mice are unreliable and unsuitable for use for classification purposes, in this instance by DECOS, owing to deficiencies in methodology and reporting. Furthermore, reliable, peer-reviewed published GLP compliant OECD/NTP Guideline studies at higher dose/exposure levels and with greater statistical power have not identified any adverse effects on male reproductive organs (5 studies, rats and mice: NTP 1997 [2 species], Murray 2014, 2019, IMOA 2016), seminology (4 studies, rat: NTP 1997, Murray 2014, 2019, Hoberman 2016) or functional fertility (2 studies, rat: Murray 2019, Hoberman 2016). **The collective weight**

		of evidence in the Expert Review and the cited multiple peer-reviewed study publications mean classification as a suspected human reproductive toxicant is not justified.

Comments on DECOS draft document on Molybdenum

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PAGE NUMBER, LINE NUMBER	COMMENT
General Comments	The overall document seems to provide sufficient information to classify Molybdenum (Mo). However, there is no adequate reference to certain mechanisms of action such as inflammation and outcomes such as cancer. The document cites IARC monograph Volume 118, which classified Mo Trioxide as 2B (Possibly carcinogenic to humans) but does not discuss the evidence (in experimental animals and mechanistic evidence) used for the carcinogenic classification. This is important to consider because Mo Trioxide is the major form of Mo in production, use, and consumption besides Sodium Molybdate.
Specific Comments	
Page 24, line 3, Section 3.1	Suggest adding the worldwide production of Mo as these numbers were available in IARC Monograph Vol. 118 which had the following citation: Polyak DE (2016). Molybdenum [advance release]. 2014. Minerals Yearbook. Washington (DC), USA: United. States Geological Survey. Maybe also tabulate Mo production industry-wide if statistics are available. Current reports can be found here: https://www.usgs.gov/centers/national-minerals-information-center/molybdenum-statistics-and-information
Page 31, Section 5	Please mention the cardiovascular effects of Mo exposure. Suggested reference: Shiue I, Hristova K [2014]. Higher urinary heavy metal, phthalate and arsenic concentrations accounted for 3-19% of the population attributable risk for high blood pressure: US NHANES, 2009–2012. Hypertens Res Dec;37(12):1075–1081. doi: 10.1038/hr.2014.121. Epub 2014 Jul 31. PMID: 25077919. https://www.nature.com/articles/hr2014121.pdf
Page 31, Section 5	Please refer to the NTP 1997 technical report (reference 86 in the draft document) for studies on chronic inflammation as one of the mechanisms of toxicity. There is limited evidence for carcinogenicity in humans through Mo exposure but there is mechanistic evidence as fibrosis and chronic inflammation were reported in experimental animals. Hence, it is important to mention these data in the mechanism section. Also, IARC monograph on Mo (Volume 118) reported sufficient evidence of cancer in experimental animals exposed to Mo. [NTP [1997]. Toxicology and carcinogenesis studies of molybdenum trioxide in F344/N rats and B6C3F$_1$ mice (inhalation studies). Research Triangle Park, NC: National Toxicology Program, TR 462. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr462.pdf. AND Ozaki K, Haseman JK, Hailey JR, Maronpot RR, Nyska A [2002]. Association of adrenal pheochromocytoma and lung pathology in inhalation studies with particulate compounds in the male F344 rat--the National Toxicology Program experience. Toxicol Pathol Mar-Apr;30(2):263–70. doi: 10.1080/01926230275355960 5. PMID: 11950170.