Reactie Gezondheidsraad op commentaar conceptadvies Molybdeen en geselecteerde anorganische molybdeen verbindingen

Response Health Council to comments draft report Molybdenum and selected inorganic molybdenum compounds

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Inhoud

1	Reactie op commentaar NIOSH	3
	Response to comments NIOSH	3
2	Reactie op commentaar IMOA	4
	Response to comments IMOA	4

1 Reactie op commentaar NIOSH

Response to comments NIOSH

Op 27 mei 2025 heeft de Gezondheidsraad per brief gereageerd op het commentaar van het NIOSH op het concept van het advies Molybdeen en geselecteerde anorganische molybdeen verbindingen. De reactie staat hieronder, in dezelfde taal als het oorspronkelijke commentaar (Engels).

On May 27th 2025, the Health Council sent a letter to the NIOSH in response to the comments on the draft report on Molybdenum and selected inorganic molybdenum compounds. The response is cited below.

Dear Dr. Chittiboyina,

Thank you for accepting the invitation to comment on the draft report 'Molybdenum and selected inorganic molybdenum compounds' that the Health Council published for public review in September 2024. I am writing in response to your comments, on behalf of the Council's Subcommittee on the Classification of Substances Toxic to Reproduction of the Dutch Expert Committee on Occupational Safety (DECOS).

The subcommittee appreciates your review of the report. In general, you made the suggestion to include additional information and references related to toxicity of action and endpoints such as cancer and cardiovascular effects. However, as the report 'Molybdenum and selected inorganic molybdenum compounds' focusses on effects on reproductive toxicity only, the subcommittee limited its evaluation to data related to effects on fertility, foetal development, and effects on or via lactation. Therefore, the title of chapter 5 has been adjusted to "Mechanism of action and mechanism of reproductive toxicity", to clarify this focus. The subcommittee agrees to including the mining locations to provide additional background information.

After careful review of incoming comments regarding the classification, the subcommittee conducted a reassessment of the available data. As a result, the classification has been adjusted. Based on the available data, the committee recommends not classifying molybdenum and selected inorganic molybdenum compounds for reproductive toxicity.

Kind regards,

Dr. R.H. Mennen
Scientific staff member

2 Reactie op commentaar IMOA
Response to comments IMOA

Op 27 mei 2025 heeft de Gezondheidsraad per brief gereageerd op het commentaar van het International Molybdenum Association (IMOA) op het concept van het advies Molybdeen en geselecteerde anorganische molybdeen verbindingen. De reactie staat hieronder, in dezelfde taal als het oorspronkelijke commentaar (Engels).
On May 27th 2025, the Health Council sent a letter to the International Molybdenum Association (IMOA) in response to the comments on the draft report on Molybdenum and selected inorganic molybdenum compounds. The response is cited below.

Dear Sandra Carey,

Thank you for accepting the invitation to comment on the draft report 'Molybdenum and selected inorganic molybdenum compounds' that the Health Council published for public review in September 2024. I am writing in response to your comments, on behalf of the Council's Subcommittee on the Classification of Substances Toxic to Reproduction of the Dutch Expert Committee on Occupational Safety (DECOS).

The subcommittee appreciates your thorough review of the report. After careful review of your comments regarding the classification, the subcommittee has conducted a reassessment of the available data. As a result of this review, the classification has been adjusted, and the committee recommends not classifying molybdenum metal or the selected inorganic molybdenum compounds as being toxic to reproduction.

The subcommittee previously assessed the two-generation study (Murray et al. 2019) and concluded that the dosing was too low for the evaluation of reproductive toxicity. Minimal or borderline parental toxicity was observed, with a 9% reduction in absolute body weight in the high-dose group compared to controls. In standard practice, a reduction of at least 10% is typically required as a threshold for parental toxicity. The subcommittee acknowledges that, at the time, selecting a dose of 40 mg/kg was reasonable, given the 15% lower absolute body weight observed in the 60 mg/kg group of the 90-day study and the approximately 10% reduction in the 40 mg/kg group of the dose-range finding study. The increased statistical power in the two-generation study, due to a larger number of animals per group, resulted in minimal or borderline parental toxicity, as the effect on absolute body weight fell below the 10% threshold. Although the subcommittee believes that dosing up to 60 mg/kg would have been more ideal based on currently available data, given the small spacing between 60 and 40 mg/kg/day and the borderline parental toxicity observed at 40 mg/kg/day, the dose level selection is considered adequate for evaluation of reproductive toxicity. The subcommittee considers this study to overrule the results of previous studies such as the ones performed by Pandey and Singh (2002) and Jeter et al. (1954). Based on these data the subcommittee concludes that a classification on reproductive toxicity is not justified. Nonetheless, the subcommittee notes that this conclusion is based on data from rodent studies only, whereas no fertility studies have been performed in non-rodent species.

The subcommittee has chosen to align with your proposal not to include insoluble or poorly soluble molybdenum or molybdenum varieties. A grouping approach based on substance solubility is usually common practice of the Dutch Health Council. In this case, it means that only the metal molybdenum was evaluated separately, as molybdenum disulfide had already been excluded, being a UVCB. Since there is a low amount of literature available on the metal molybdenum, the subcommittee concludes that the metal cannot be classified due to lack of appropriate available data. Because tetrathiomolybdates are considered to have a different working mechanism than the other molybdates, they are no longer included in the advisory report.

The subcommittee's common practice is to use ECHA conversion factors for the calculation of dose levels, and not to use EFSA conversion factors. However, when sodium molybdate was tested, the subcommittee did not consistently calculate the molybdate concentration using the 40% conversion factor. This has now been adjusted in the advisory report. This adjustment does not have consequences for the hazard assessment.

Other specific comments that were addressed in the third part of your response are addressed in the enclosed annex.

Kind regards,

Dr. R.H. Mennen
Scientific staff member

Response to IMOA Comments on: Molybdenum and selected inorganic molybdenum compounds, Health Council of the Netherlands: DRAFT, Sept 2024

1 **Page/ Line No**	**IMOA Comment:**	**Comment Health Council:**
Executive Summary		
8/5	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.	The summary was adjusted according to the adjustments made in the advisory report.
8/21-24	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	The summary was adjusted according to the adjustments made in the advisory report.
2. Identity of the substance		
13/8 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.	The subcommittee agrees the data on the metal are very scarce. However, in the report the subcommittee has included a study on molybdenum nanoparticles and for this reason molybdenum is included in this table. Mohamed HRH, El-Atawy RH, Ghoneim AM and El-Ghor AA. *Induction of fetal abnormalities and genotoxicity by molybdenum nanoparticles in pregnant female*

		mice and fetuses. Environ Sci Pollut Res Int 2020; 27(19): 23950-62.
13/8-9 tables 1/2	"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. 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, (NH4)3PMo12O40 or ammonium tetrathiomolybdate, (NH4)2MoS4. 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, (NH4)4Mo8O26, which is tetraammonium hexacosaoxo octamolybdate. The hexacosaoxo indicates the 26 (hexacosa) oxygen atoms in the formula (NH4)4Mo8O26. 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; oxygen(2-); hydrate / 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 anhydrous form. Therefore, the EC number 601-720-3 is obsolete. 603-021-9 / 12501-45-0 / Molybdate (Mo7O246-), ammonium (1:6) / This is likely an old/redundant entry in the EC inventory for hexaammonium heptamolybdate.	The committee has adjusted the name 'tetraammonium hexamolybdate' to 'tetraammonium octamolybdate' in tables 2, 3, and 4 where applicable. The EC name was retained to tetraammonium hexamolybdate as it has been registered that way, but a note has been added to mention this name is incorrect. The substances ammonium molybdate could be confused with, were not included in the advisory report. The one study that concerns ammonium molybdate is Howell 1993, which was a poorly reported study that did not include the CAS-number. Ammonium molybdate may be confused with ammonium paramolybdate, but this one is clearly mentioned separately in the table and has a different CAS-number.

13/12-14	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.	The subcommittee has chosen to align with your proposal to not include insoluble or poorly soluble molybdenum or molybdenum varieties, as is usually also common practice by the Dutch Health Council. In this case it means that only the metal Molybdenum will be evaluated separately, as molybdenum disulfide was already excluded being a UVCB. This is clarified in section 2.1. Since there is a low amount of literature available on the molybdenum metal, the subcommittee evaluates that the metal cannot be classified because of a lack in appropriate available data.
15/Table 1	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.	This footnote indeed does not apply to any of the substances named in the table and so the footnote has been removed.
16/Table 2	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.	Ammonium tetrathiomolybdate has been removed from the list. This is clarified in section 2.1.
19/Table 3	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.	See earlier response to the comment on ammonium molybdate. Ammonium tetrathiomolybdate has been deleted.
2.3 International classifications		
21/17-18	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."	The information is now updated to the newest version of the list of MAK and BAT values from 2024, that no longer includes a category 3B.
21/29-31 22/1-3 Table 4	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	The subcommittee is aware the NITE evaluated molybdenum (7439-98-7), sodium molybdate (7631-95-0), ammonium molybdate (12027-67-7) in 2015 and molybdenum trioxide

	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	(1313-27-5) in 2019. This is clarified in section 2.3.2.
22/Table 4	See above on unambiguous substance identification, specifically on the ammonium molybdates. Thiomolybdates should not be considered.	The thiomolybdate has been deleted from this table.
3. Manufacture and Uses		
24/26-27	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.	A clarification has been added to the last sentence of this paragraph: “… molybdenum trioxide also known as technical grade molybdenum oxide.”
4. Toxicokinetics		
26/27	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.	Hydroxides has been removed.
26/28	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	This statement has been removed.

	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	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.	The sentence is adjusted to: "..using split-thickness human skin membranes" following the description in the REACH dossier. Also the corresponding molybdate concentrations as mentioned in the REACH dossier are added.
30/10-13	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.	This statement has been removed.
5. Mechanism of action and toxicity		
31/11-13	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.	An adjustment has been made accordingly.
31/29 - 33 32/1-9	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 guidelines. While they can be useful adjuncts for investigating possible	The committee carefully phrased it as being '.. a potential association between molybdenum and oxidative stress.' The committee is not suggesting in this section that markers of oxidative stress on their own would be indicators of testicular toxicity, yet the committee only is mentioning papers have studied this and correlations and associations may exist. Phrasing has been adjusted to make this more clear.

	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.	The sentence including the reference to Domingo-Relloso et al [2019], Ref 66, has been removed since it concerned tetrathiomolybdates which are no longer included in this advisory report. The committee has evaluated the reference of Joun et al. (2024).This study focused on the association between molybdenum and kidney disease rather than reproductive outcomes. The study also evaluated oxidative stress outcomes. The cross-sectional design (a study based on NHANES data) limits causal interpretations. The study assessed urinary molybdenum levels in relation to oxidative stress and inflammation markers, but it is unclear how these observed exposure levels compare to higher occupational exposures. The observed molybdenum concentrations appear relatively low. These could plausibly be associated with reduced oxidative stress. Given the mixed evidence on associations between molybdenum exposure and oxidative stress, further research is needed to clarify these associations. For these reasons and the fact that the publication date was after the committee's literature search date, the committee has decided to not make a reference to this paper.
6.1 Human data		
33/18-24, 34/5-13	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 authors used NHANES cross-sectional data, limiting the ability to draw causal inferences. While the study included a larger dataset than previous research, issues remain with statistical modelling and presentation of results. The paper evaluates two machine learning approaches to assess which factors are most predictive of the outcome, testosterone. Results of a multivariable regression from backwards selection. These are not optimal approaches for assessing

	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	associations between an exposure of interest and a health outcome. They can be prone to overfitting and/or misspecification and modelling of covariates. It would have been preferable if assumed relationships between covariates (including confounders, mediators, colliders) had been specified, for example using a directed acyclic graph (DAG). The machine learning methods were presented in a somewhat 'black box' way; more insight into the change in effect estimates upon adjustment (e.g., minimally and further adjusted confounder models) on model selection and stability and scaling of variables would have been preferable. Further, there are other approaches for assessing exposure-outcome relationships using more advanced (multiple exposure) statistical modelling approaches that have been assessed in simulation modelling and are more accepted in the field of (environmental) epidemiology. In summary, the findings should be interpreted with caution, also given the authors affiliations, and speculative explanations should be avoided. Additionally, the publication date was after the committee's literature search date. The committee has decided to not include this paper in the advisory report.
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	**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 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	An additional sentence has been added.

	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	**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	The committee agrees the study was too poorly reported to make a definite conclusion.

	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. Jean-Faucher C, Berger M, de Turckheim M, Veyssière G, Jean C. 1982, The effect of preweaning undernutrition upon the sexual development of male mice. Biol Neonate. 41(1-2):45-51. doi:10.1159/000241515 Schneider JE. Energy balance and reproduction. Physiology and Behavior. 2004;81(2):289–317. doi: 10.1016/j.physbeh.2004.02.007 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. Rugh R. 1968.The Mouse: Its Reproduction and Development. (Oxford University Press, New York). ISBN 10: 0198542771 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 [PubMed: 23522086] Zorrilla EP. 1997. Multiparous species present problems (and possibilities) to developmentalists. Dev Psychobiol. 30(2):141-50. doi: 10.1002/(sici)1098-	

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37/4 - 16	**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	An additional sentence has been added.

	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	**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	The committee is aware that guinea pigs have disadvantages for reproductive toxicology studies and did not attach a lot of weight to this study in the overall weight of evidence.

	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	**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.	See the general comment in the response letter.

	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	**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 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	According to the article, dose levels of 17 instead of 7 mg Mo/kg bw/day were administered. See the general comment in the response letter.

	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	**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	All the assessed readouts were summarised in the supplementary table.

	studies [Murray et al 2014, 2019; Aveyard 2023, pages 162,164 and 187]	
41/3-5 41/9-10	**Dose levels: 0, 3, 10, 30 and 100 mg MoO3/m3 5 days/week, 6.5h/day** *See attached Expert Review.* This publication reports a programme of inhalation studies with MoO3 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 MoO3/m3, 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 MoO3/m3 providing results similar to the 30 mg MoO3/m3 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.	The committee agrees no adverse effects on the reproductive organs or sperm parameters were found.
41/11-33	**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	An evaluation by the committee is added to this paragraph. Also see the general comment in the response letter.

	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. 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	

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41/34-35 42/1-9 Page 115	**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).	See the general comment in the response letter.
42/10-32	**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.	See the general comment in the response letter.
43/1-10	**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	A sentence has been added to this paragraph.

	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. 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	**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, 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	A sentence has been added to this paragraph.

	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	**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	The committee clearly described various reasons why this study was poorly reported.

	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.** 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	**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	The committee considered the doses as applied as too low to observe relevant effects.

	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.**	
6.3 Data Evaluation		
44/1-11	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.	See the general comment in the response letter.
6.4 Conclusion		
48/22-26	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.**	See the general comment in the response letter.