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Public draft

Gezondheidsraad

Molybdenum and selected inorganic molybdenum compounds

Evaluation of the effects on reproduction, recommendation for classification

Subcommittee on the Classification of Substances Toxic to Reproduction
A Committee of the Health Council of the Netherlands

The Health Council would like to give you the opportunity to comment on the draft advisory report. The draft has been presented to the Working Conditions Committee of the Social Economic Council of the Netherlands, and to experts of employer's organisations and trade unions. Other interested parties or persons are also invited to comment. The comments will be taken into account when drafting the final version of the advisory report.

Please follow the instructions for review, see www.healthcouncil.nl.

Please note that this is a draft report that will be finalised after comments received during public consultation have been considered. When citing from this report, please indicate that you are citing from a draft version.

Comments may be submitted until **December 2, 2024**
By e-mail: draftOSH@gr.nl

Attn: Dr. R.H. Mennen
Subcommittee on the Classification of Substances Toxic to Reproduction
The Health Council of The Netherlands

Contents

Samenvatting

Werknemers kunnen tijdens het werk worden blootgesteld aan stoffen die mogelijk schadelijk zijn voor hun gezondheid. Op verzoek van de minister van Sociale Zaken en Werkgelegenheid (SZW) heeft de Gezondheidsraad beoordeeld of molybdeen en geselecteerde anorganische molybdeenverbindingen schadelijke eigenschappen hebben die invloed kunnen hebben op de voortplanting, en op basis daarvan een classificatievoorstel opgesteld.
Dit advies is tot stand gekomen in de subcommissie Classificatie reproductietoxische stoffen, van de commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS). Op www.gezondheidsraad.nl staat informatie over de taken van deze vaste subcommissie van de Gezondheidsraad. De samenstelling van de subcommissie is te vinden achterin dit advies.

Gebruik van molybdeen
Molybdeen is een mineraal spoorelement dat wijdverspreid in de natuur voorkomt. Het heeft een essentiële biologische functie als micronutriënt in verschillende planten en dieren, maar ook in mensen. Molybdeen wordt ook gebruikt in de metaalindustrie, bijvoorbeeld bij de productie van gietijzer en roestvrij staal. De algemene bevolking wordt vooral blootgesteld aan molybdeen via voeding. Beroepsmatige blootstelling komt voor als molybdeen tijdens industriële processen vrijkomt in de lucht.

Classificeren naar bewijskracht
Bij de beoordeling van het effect op de voortplanting kijkt de commissie zowel naar de effecten op de vruchtbaarheid van mannen en vrouwen als naar de effecten op de ontwikkeling van het nageslacht. Daarnaast worden de effecten op de lactatie (productie en afgifte van moedermelk) beoordeeld en de effecten via de moedermelk op de zuigeling.
Als er aanwijzingen bestaan dat de stof schadelijke effecten heeft, stelt de commissie voor om de stof in te delen in gevarencategorieën die aangeven hoe groot de bewijskracht is voor de schadelijke effecten, zie kader. Bij categorie 1 is de bewijskracht het grootst en grotendeels gebaseerd op studies bij mensen (1A) of dieren (1B). Bij categorie 2 is de bewijskracht beperkt en is er sprake van een ‘verdenking’. De commissie kan ook adviseren om een stof niet te classificeren omdat er onvoldoende gegevens beschikbaar zijn of omdat de stof waarschijnlijk niet schadelijk is voor de voortplanting.

Een classificatievoorstel zegt iets over de bewijskracht voor de schadelijke eigenschappen van een stof, maar niet over de mate waarin mensen op de werkplek een gezondheidsrisico lopen. Dat hangt namelijk af van de mate waarin mensen op hun werk worden blootgesteld aan de stof. Daar heeft de commissie geen zicht op.

Geraadpleegde onderzoeken

Naar het effect van blootstelling aan molybdeen en geselecteerde anorganische molybdeenverbindingen op de vruchtbaarheid zijn verschillende onderzoeken gedaan bij mensen. De gegevens uit deze onderzoeken acht de commissie onvoldoende overtuigend. Onderzoeken bij dieren laten zien dat blootstelling aan molybdeen een nadelig effect heeft op de kwaliteit van het sperma van mannelijke ratten en muizen. Er kan geen algemene conclusie worden getrokken over de invloed hiervan op de vruchtbaarheid, omdat daar geen geschikte data over beschikbaar was. De doseringsniveaus waren te laag en de resultaten bij vergelijkbare doseringsniveaus waren tegenstrijdig. Daarom adviseert de commissie om molybdeen en geselecteerde anorganische molybdeenverbindingen te classificeren in categorie 2 (kan mogelijk de vruchtbaarheid schaden).

De commissie concludeert dat er op basis van de beschikbare gegevens geen reden is voor classificatie van molybdeen en geselecteerde anorganische molybdeenverbindingen voor de ontwikkeling van het nageslacht. Deze conclusie is echter alleen gebaseerd op onderzoek bij knaagdieren. Er zijn nog geen ontwikkelingsstudies met niet-knaagdieren beschikbaar.

Er zijn onvoldoende relevante onderzoeksgegevens beschikbaar om een conclusie te trekken wat betreft het effect van molybdeen en geselecteerde anorganische molybdeenverbindingen op of via de lactatie.

Advies aan de minister

Op basis van de beschikbare onderzoeksgegevens adviseert de commissie om molybdeen en geselecteerde anorganische molybdeenverbindingen:

- te classificeren als een stof die ervan verdacht wordt schadelijk te zijn voor de vruchtbaarheid (categorie 2) en te kenmerken met H361f (verdacht van het schaden van vruchtbaarheid);
- niet te classificeren voor effecten op de ontwikkeling van het ongeboren kind omdat de beschikbare onderzoeksgegevens hiervoor geen aanleiding geven;
- niet te classificeren voor de effecten op of via lactatie omdat er onvoldoende relevante onderzoeksgegevens zijn.

Betekenis classificatievoorstellen reproductietoxische stoffen

In classificatievoorstellen gebruikt de Gezondheidsraad een indeling in gevarencategorieën. De categorieën zijn afgeleid van EU-verordening (EG) 1272/2008 en geven aan hoe sterk de bewijskracht is voor schadelijke effecten. Bij de categorie hoort ook een label met een EU-gevarenaanduiding die op verpakkingen kan worden gebruikt.

EU-gevarencategorieën voor voortplanting (*fertility* – F) en ontwikkeling (*development* – D)

• Categorie 1: Kan de vruchtbaarheid of het ongeboren kind schaden (EU-gevarenaanduiding H360 F/D)

• Categorie 1A: Stoffen waarvan bekend is dat zij toxisch zijn voor de menselijke voortplanting, hoofdzakelijk gebaseerd op onderzoek bij mensen (H360 F/D).

• Categorie 1B: Stoffen waarvan verondersteld wordt dat zij toxisch zijn voor de menselijke voortplanting (hoofdzakelijk gebaseerd op dierstudies (H360 F/D).

• Categorie 2: Kan mogelijk de vruchtbaarheid of het ongeboren kind schaden (H361f/d) - Stoffen die ervan verdacht worden dat zij toxisch zijn voor de menselijke voortplanting

EU-gevarencategorie voor effecten op of via lactatie

• Kan schadelijk zijn via de borstvoeding (H362). Stoffen waarvan is aangetoond dat zij de lactatie beïnvloeden of die in zodanige hoeveelheden in moedermelk aanwezig kunnen zijn dat er reden is tot bezorgdheid voor de gezondheid van het kind dat borstvoeding krijgt.

Betekenis voor de werkvloer

Werkgevers zijn op grond van de Arbowet wettelijk verplicht om gezondheids- en veiligheidsrisico's van het werken met stoffen zoveel mogelijk te voorkomen of te beperken. Op basis van de classificatievoorstellen van de Gezondheidsraad kan de minister van SZW besluiten stoffen op te nemen in de officiële lijst van kankerverwekkende, mutagene en voor de voortplanting giftige stoffen. Op die lijst staan kankerverwekkende en mutagene stoffen in categorie 1A en 1B en voor de voortplanting giftige stoffen in categorie 1A, 1B en 2. Afhankelijk van de classificatie vraagt de wetgever de werkgever aanvullende maatregelen te nemen om de werknemer te beschermen.

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Executive summary

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluated the effects of molybdenum and selected inorganic molybdenum compounds on reproduction. Based on this evaluation they made a recommendation for classification. This advisory report was drafted by the subcommittee on the Classification of Substances Toxic to Reproduction of the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council, hereafter called the committee.

The Health Council has a permanent task in assessing the hazard of substances to which workers can be occupationally exposed. More information about this task can be found at www.gezondheidsraad.nl.

Use of molybdenum

Molybdenum is a naturally existing trace element widely distributed in nature. It serves an essential biological function as a micronutrient in various plants and animals, and also in humans. Molybdenum is widely used in the metal industry, for example in the production of cast iron and stainless steel. For the general population, the main route of exposure to molybdenum is through the consumption of food. Occupational exposure can occur when molybdenum is released into the air during industrial processes.

Classification based on strength of evidence

To assess effects on reproduction, the committee evaluates the available literature on the effects on male and female fertility and on the development of offspring. Moreover, the committee considers effects of a substance on lactation and on the offspring via lactation. If the data indicate hazardous properties, the committee recommends classification into a hazard category. Classification is performed according to EU-regulation (EC) 1272/2008 (see text box).

When there are indications that a substance has hazardous properties, the committee recommends classifying the substance into hazard categories that indicate the strength of the evidence for hazardous effects (see text box). For category 1, the strength of evidence is highest, and largely based on studies in humans (1A) or animals (1B). For category 2, evidence is limited and the substance is categorised as a ‘suspected toxicant’. The committee can also recommend not classifying a substance because of insufficient data or because the substance is probably not hazardous for reproduction.

A recommendation for classification reflects the strength of evidence for the hazardous properties of a substance, but it does not reflect the health risk for workers. The health risk is based on the level of exposure to the substance in the workplace. The committee does not have sufficient data on these exposure levels.

Reviewed literature

The available human data provided no sufficient evidence for classification for adverse effects of molybdenum or molybdenum compounds on sexual function and fertility. 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 dose levels used in the studies were considered to be low and the results at similar dose levels were contradictory. Therefore, the committee recommends classifying molybdenum and selected inorganic molybdenum compounds into category 2 (Suspected human reproductive toxicant).

The committee concludes that based on the available data on rodents, there is no reason to classify molybdenum and molybdenum compounds for developmental toxicity. However, this conclusion is based on data from rodent studies, as no developmental studies have been performed in non-rodent species.

The committee found no relevant scientific data to draw conclusions on an adverse effect of exposure to molybdenum or molybdenum compounds on or via lactation.

Recommendations to the Minister

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);
- not to classify molybdenum and selected inorganic molybdenum compounds for developmental toxicity, because the available data do not justify classification;
- not to classify molybdenum and selected inorganic molybdenum compounds for effects on or via lactation due to a lack of relevant data.

Classification for substances toxic to reproduction
The Health Council performs classification and labelling of substances according to the guidelines of the European Union (Regulation (EC) 1272/2008). The hazard categories described below indicate the strength of the evidence for hazardous properties of the substance. The substance is also labelled with an EU hazard statement code that can be used on packaging. **Classification for reproduction (fertility (F) and development (D)):** •Category 1: Known or presumed human reproductive toxicant - Causes adverse effects on fertility or the unborn child (Hazard statement code H360(F/D)). •Category 1A: Known human reproductive toxicant – Substances that are known to be toxic for human reproduction, largely based on human studies (H360 (F/D)).

•Category 1B: Presumed human reproductive toxicant – Substances that are presumed to be toxic to human reproduction, largely based on animal studies (H360 (F/D)).
•Category 2: Suspected human reproductive toxicant – Can possibly affect fertility or the unborn child. Evidence from animal and/or human studies is limited (H361(f/d)).

Classification for lactation:

- Effects on or via lactation (H362) – Substances which have been proven to affect lactation or which are present in breast milk in such quantities that there is reason for concern for the health of the breastfed child.

Implications for the workplace

According to the Dutch Working Conditions Act, employers are legally required to prevent or minimize the health and safety risks of working with hazardous substances as much as possible. Based on the Health Council's recommendations for classification, the Minister of Social Affairs and Employment can decide to add substances to the official list of substances that are carcinogenic, mutagenic or toxic to reproduction. This list includes carcinogenic and mutagenic substances in categories 1A and 1B, and substances toxic to reproduction in categories 1A, 1B and 2. Depending on the classification, the government asks the employer to take additional measures to protect employees.

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1 Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. This classification is performed by the Health Council's Subcommittee on the Classification of reproduction toxic substances of the Dutch Expert Committee on Occupational Safety (DECOS). The classification is performed according to European Union Regulation (EC) 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. The CLP regulation is based on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS). The subcommittee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as reproductive toxicant (category 1A and 1B and 2) or compound with effects on or via lactation.

1.2 Committee and procedure

This document contains the recommendations for classification of molybdenum and selected inorganic molybdenum compounds by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances, hereafter called the committee. The members of the committee are listed on the last page of this report. The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and offspring development as well as adverse effects on or via lactation.

Classification for reproduction (fertility (F) and development (D)):

- Category 1 Known or presumed human reproductive toxicant (H360(F/D))
- Category 1A Known human reproductive toxicant
- Category 1B Presumed human reproductive toxicant
- Category 2 Suspected human reproductive toxicant (H361(f/d))
- No classification for effects on fertility or development

Classification for lactation:

- Effects on or via lactation (H362)
- No labelling for lactation

Hazard statement codes:

H360F May damage fertility.
H360D May damage the unborn child.
H361f Suspected of damaging fertility.

H361d	Suspected of damaging the unborn child.
H360FD	May damage fertility. May damage the unborn child.
H361fd	Suspected of damaging fertility. Suspected of damaging the unborn child.
H360Fd	May damage fertility. Suspected of damaging the unborn child.
H360Df	May damage the unborn child. Suspected of damaging fertility.
H362	May cause harm to breast-fed children.

Additional considerations to Regulation (EC) 1272/2008
If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the offspring, the compound will be classified in category 1A, irrespective of the general toxic effects (see Regulation (EC) 1272/2008, 3.7.2.2.1.). Adverse effects in a reproductive study, reported without information on the paternal or maternal toxicity, may lead to a classification other than category 1B, when the effects occur at dose levels which cause severe toxicity in general toxicity studies. Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se. The committee does not only use guideline studies (studies performed according to OECD (Organisation for Economic Cooperation and Development) standard protocols) for the classification of compounds, but non-guideline studies are taken into consideration as well.

The classification and labelling of substances is performed according to the guidelines of the European Union (Regulation (EC) 1272/2008). The classification of compounds is the result of an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The guideline necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the regulation, the committee has agreed upon a number of additional considerations.

Regarding fertility, the committee considers data on parameters related to male and female fertility, such as seminal fluid volume and spermatozoa concentration, that are related to male fertility. The committee excludes publications containing only data on sex hormone levels from the assessment, because the relationship between these hormone levels and functional fertility (ability to conceive children) is too uncertain.

In 2024, the President of the Health Council released a draft of the report for public review. The committee has taken the comments received into account in deciding on the final version of the report. These comments, and the replies by the committee, can be found on the website of the Health Council.

1.3 Labelling for lactation

The recommendation for classifying substances for effects on or via lactation is also based on Regulation (EC) 1272/2008. The criteria define that substances which are absorbed by women and have been shown to interfere with lactation or which may be

present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, should be classified and labelled. Unlike the classification of substances for fertility and developmental effects, which is based on hazard identification only (largely independent of dosage), the labelling for effects on or via lactation is based on a risk characterization and therefore, it also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects on or via lactation when it is likely that the substance would be present in breast milk at potentially toxic levels. The committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration leads to exceeding the exposure limit for children, or if that level is unknown, the exposure limit for the general population, e.g. the acceptable daily intake (ADI).

1.4 Data

Information regarding reproductive toxicity of molybdenum and selected inorganic molybdenum compounds were evaluated with the DECOS report on molybdenum compounds from 2013 as a starting point. A new literature search was performed for publications from 2013 until February 2023 (search terms see annex B). The studies described in the 2013 DECOS report, along with new literature, were evaluated, and their summaries are included in this classification.

2 Identity of the substance

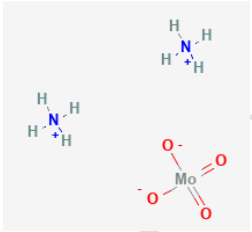
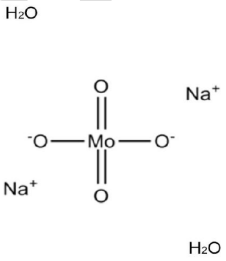
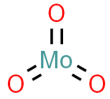
2.1 Name and other identifiers of the substance

This overview summarizes information on molybdenum and a selection of molybdenum compounds. The main sources used were a report of The Dutch Expert Committee on Occupational Safety (a committee of the Health Council of the Netherlands), a report by the ATSDR, the *Handbook of chemistry and physics* and ECHA's database of registrations.[1-4] 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). Since data on reproduction toxicity for other molybdenum compounds were lacking (Table 2), the committee applied a group approach. The identity and physicochemical properties of molybdenum and selected molybdenum compounds are given below (Table 1 and 2).The group approach is based on the ECHAs Read-Across Assessment Framework of metal compounds (according to RAAF Scenario 3).[5] In short, the main assumption underlying grouping of metal compounds in this approach is that toxicological properties are likely to be similar or follow a similar pattern as a result of the presence of a common metal ion. In this case, the common metal ion is the molybdate ion, MoO_4^{2-} that is responsible for systemic effects, and to which the molybdenum compounds (bio)transform in water and in body fluids (as outlined under Section 2.3). Thus, systemic toxicity correlates with the ability of the substance to release molybdate ions that can then be absorbed by the body. Systemic toxicity is generally lower for less soluble/bio-accessible substances. A worst-case approach is applied, in which the committee assumes that the systemic toxicity data concerning molybdate are applicable to other mono-constituent molybdenum compounds.

Next to the mono-constituent molybdenum substances, more complex structures containing molybdenum exist, such as reaction products, UVCBs and mixtures. The committee decided to not use these complex structures for grouping and those were therefore excluded from further selection.

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.

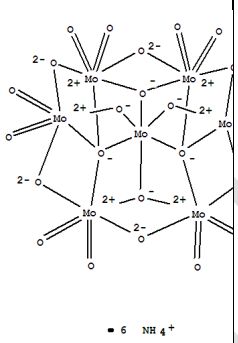
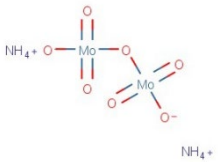
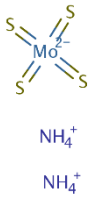
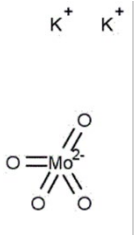
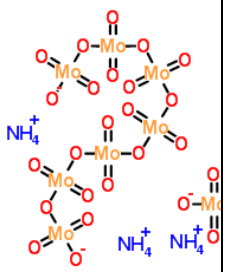
Name(s) in the IUPAC nomenclature or other international chemical name(s)	Molybdenum	Ammonium molybdate(VI)	Disodium dioxomolybdenumbis (olate)	Molybdenum trioxide
Other names (usual name, trade name, abbreviation)		Diammonium molybdate; Ammonium orthomolybdate	Sodium molybdate, disodium molybdate	
ISO common name (if available and appropriate)	N/A	N/A	N/A	N/A
EC/EINECS number (if available and appropriate)	231-107-2	236-031-3	231-551-7	215-204-7; 231-970-5 (molybdic acid; hydrate)
EC name (if available and appropriate)	Molybdenum	Ammonium molybdate(VI)	Disodium molybdate	Molybdenum trioxide; molybdic acid (hydrated forms)
CAS number	7439-98-7	13106-76-8	7631-95-0; 10102-40-6 (dihydrate)	1313-27-5; 7782-91-4, 25942-34-1 (molybdic acid hydrates)
SMILES code (if available)	[Mo]	[NH4+].[NH4+]. [O-][Mo](=O) (=O)[O-]	O.O.[O-][Mo](=O)(=O) [O-].[Na+].[Na+]	O=[Mo](=O)=O

Molecular formula	Mo	$(NH_4)_2MoO_4$	Na_2MoO_4 ($Na_2MoO_4.2H_2O$)	MoO_3 $MoO_3 \cdot H_2O$ (hydrate) $MoO_3 \cdot 2H_2O$ (dihydrate)
Structural formula	Mo			
Molecular weight or molecular weight range	95.96	196.01	205.92; 241.95 (dihydrate)	143.94

[a] Substance consists for ≥45— ≤96% (w/w) of MoO_3, and to a lesser extent SiO_2, Mo suboxides, MoO_2, iron molybdates, lead molybdate, arsenic oxide, copper molybdate, calcium molybdate and ammonium molybdates.

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.

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Hexaammonium heptamolybdate tetrahydrate	diammonium [(oxidodioxomolybdenio)oxy]molybdenum oylolate	Bisammonium tetrakis(sulfido)molybdate(2-)	Dipotassium tetraoxomolybdenum diuide	Tetraammonium bis (dioxomolybdenumbis (olate))
Other names (usual name, trade name, abbreviation)	Ammonium molybdate (VI) tetrahydrate; ammonium paramolybdate	Diammonium dimolybdate	Ammonium tetrathio molybdate	Dipotassium tetraoxomolybdate; potassium molybdate	Tetraammonium hexamolybdate; ammonium octamolybdate
ISO common name (if available and appropriate)	N/A	N/A	N/A	N/A	N/A
EC/EINECS number (if available and appropriate)	234-722-4	248-517-2	640-219-4	236-599-2	235-650-6
EC name (if available and appropriate)	N/A	Diammonium dimolybdate		Dipotassium tetraoxomolybdate	Tetraammonium hexamolybdate
CAS number	12027-67-7; 12054-85-2 (tetrahydrate)	27546-07-2	15060-55-6	13446-49-6	12411-64-2
SMILES code (if available)	[NH4+].[NH4+].[NH4+].[NH4+].[NH4+].[NH4+].[O-][Mo] (=O)(=O)[O-].[O-][Mo](=O) (=O)[O-	[NH4+].[NH4+]. [O-][Mo](=O)(=O)O[Mo]([O-])(=O)=O	[NH4+].[NH4+].S=[Mo--] (=S)(=S)=S	[K+].[K+].O=[Mo--](=O)(=O)=O	[NH4+].[NH4+].[NH4+].[NH4+].[O-][Mo](=O)(=O)[O-].[O-][Mo](=O)(=O)O[Mo]

	].[O-][Mo](=O)(=O)[O-]				(=O)(=O)O[Mo](=O)(=O)O[Mo](=O)(=O)O[Mo](=O)(=O)O[Mo](=O)(=O)O[Mo](=O)(=O)O[Mo](=O)(=O)[O-]
Molecular formula	$(NH_4)_6Mo_7O_{24}$ $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	$(NH_4)_2Mo_2O_7$	$H_8MoN_2S_4$	K_2MoO_4	$(NH_4)_4Mo_8O_{26}$
Structural formula					
Molecular weight or molecular weight range	1163.80 1235.86 (tetrahydrate)	339.95	260.28	238.14	1255.66

2.2 Physicochemical properties

The physicochemical properties of molybdenum and its compounds are presented in Table 3. The ECHA dissemination website and the *Handbook of chemistry and physics* were used as the primary source.[1, 3]

Molybdenum (Mo) is a naturally occurring metallic trace element found in natural minerals, but not as the free metal. Biologically, it is an important micronutrient in plants and animals, including humans. It is used widely in industry for metallurgical applications (See also Section 3.2).[2]

Molybdenum has oxidation states from -2 to +6. Commonly encountered compounds are those of molybdenum in oxidation state +6 (Mo(VI), MoO_3, molybdates) and +4 (Mo(IV), MoS_2).[2]

Molybdenum (VI) anions include molybdate (MoO_4^{2-}) and polymeric anions (isopolymolybdates) of which the most common are heptamolybdate ($Mo_7O_{24}^{6-}$) and octamolybdate ($Mo_8O_{26}^{4-}$). These anions occur in sodium and ammonium salts, often hydrated, which are the common sources of molybdenum in commerce and industrial applications.[2]

Under physiological conditions (pH >6.5), the molybdate anion, MoO_4^{2-}, is the sole molybdenum species in aqueous media.

Table 3. Summary of physicochemical properties of molybdenum and its selected compounds.

Substance	**State of the substance at normal temperature and pressure**	**Melting/freezing point (at 101325 Pa)**	**Boiling point (at 101325 Pa)**	**Relative density (at 20°C)**	**Water solubility**
Molybdenum	Solid	2,623°C	4,639°C	10.18	Insoluble
Ammonium molybdate(VI)	Solid	>190°C [b]	N/A [a]	1.4	Soluble: 10 g/L
Disodium molybdate	Solid	687°C	N/A [a]	2.59	Soluble: 654 g/L
Molybdenum trioxide	Solid, powder, white-yellow to bluish, odourless, inorganic.	802°C	1,155°C	4.66	Slightly soluble: 1 g/L
Ammonium paramolybdate	Solid	>90°C [b]	N/A [a]	2.86	Soluble: 206.5 g/L [c]
Diammonium dimolybdate	Solid, powder, white to greyish,	>150°C [b]	N/A	2.97	Soluble: 228 g/L

Substance	**State of the substance at normal temperature and pressure**	**Melting/freezing point (at 101325 Pa)**	**Boiling point (at 101325 Pa)**	**Relative density** **(at 20°C)**	**Water solubility**
	odourless, inorganic.				
Ammonium tetrathio molybdate [a]	Solid	>100°C [b]	-	-	Slightly soluble
Dipotassium tetraoxomolybdate	Solid fine white powder	926°C	N/A [b]	3.09	Soluble: 183 g/100 g H_2O
Tetraammonium hexamolybdate	Solid, crystalline, white, odourless, inorganic	>287°C [b]	N/A	3.74	Slightly soluble: 1 g/L

[a] No REACH registration dossier available
[b] Decomposes before melting.
[c] Solubility for CAS number 12054-85-2, according to the CRC handbook: 43 g/100 ml H_2O

2.3 International classifications

2.3.1 European Commission

Molybdenum trioxide has a harmonized classification for three hazard classes:

- Eye Irrit. 2 (H319: causes serious eye irritation)
- STOT SE 3 (H335: may cause respiratory irritation)
- Carc. 2 (H351: suspected of causing cancer)

The other selected molybdenum compounds and molybdenum do not have a harmonized classification under the European CLP regulation.

2.3.2 Other countries

None of the 9 selected molybdenum compounds have been classified for reproductive toxicity in Australia.[6] Molybdenum trioxide and its hydrated form molybdenic acid have been classified in Australia for carcinogenicity (category 2), eye irritation (category 2A) and specific target organ toxicity - single exposure (category 3), in accordance with GHS.

In Germany, none of the selected molybdenum compounds have been included in the list of additional carcinogenic, mutagenic and reprotoxic (CMR) substances in the context of worker protection.[7] However, molybdenum trioxide has been classified in carcinogen category 3B by the German Research Foundation (DFG). Category 3 includes substances for which in vitro or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories.

According to the IARC, molybdenum trioxide is possibly carcinogenic to humans (Group 2B). The other selected molybdenum compounds have not been evaluated by IARC.

In the state of California, molybdenum trioxide has been listed as a carcinogen since 19 March 2021.[8] The other selected molybdenum compounds have not been included in this list. The selected molybdenum compounds have not been included in the Report on Carcinogens (15th edition),[9] or in the NIOSH carcinogen list.[10]

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.

Table 4. Classification of molybdenum and selected inorganic molybdenum compounds in Japan.

Compound (CAS number)	**GHS classification in Japan**
Molybdenum[11] (7439-98-7)	Skin Irrit. 2 (H315: Causes skin irritation) Eye Irrit. 2 (H319: Causes serious eye irritation) STOT SE 3 (H335: May cause respiratory irritation)
Ammonium molybdate (VI) (13106-76-8)	-
Sodium molybdate[12] (7631-95-0)	Acute Tox. 3 (H301: Toxic if swallowed) Skin Irrit. 2 (H315: Causes skin irritation) Eye Irrit. 2 (H319: Causes serious eye irritation) Muta. 2 (H341: Suspected of causing genetic defects) Carc. 2 (H351: Suspected of causing cancer) Repr. 2 (H361: Suspected of damaging fertility or the unborn child) STOT SE 3 (H335: May cause respiratory irritation) STOT RE 1 (H372: Causes damage to organs through prolonged or repeated exposure (systemic toxicity, testis)) STOT RE 2 (H373: May cause damage to organs through prolonged or repeated exposure (kidney))
Sodium molybdate dihydrate (10102-40-6)	-
Ammonium paramolybdate[13] (12027-67-7)	Acute Tox. 4 (H302: Harmful if swallowed) Eye Irrit. 2 (H319: Causes serious eye irritation) Carc. 2 (H351: Suspected of causing cancer) Repr. 2 (H361: Suspected of damaging fertility or the unborn child) STOT SE 3 (H335: May cause respiratory irritation) STOT RE 2 (H373: May cause damage to organs through prolonged or repeated exposure (kidney))
Ammonium paramolybdate tetrahydrate (12054-85-2)	-

Compound (CAS number)	**GHS classification in Japan**
Diammonium dimolybdate (27546-07-2)	-
Ammonium tetrathiomolybdate (15060-55-6)	-
Potassium molybdate (13446-49-6)	-
Molybdenum trioxide[14] (1313-27-5)	Eye Irrit. 2 (H319: Causes serious eye irritation) Carc. 2 (H351: Suspected of causing cancer) Repr. 2 (H361: Suspected of damaging fertility or the unborn child) STOT SE 3 (H335: May cause respiratory irritation) STOT RE 1 (H372: Causes damage to organs through prolonged or repeated exposure (respiratory organs, reproductive organs male)) STOT RE 2 (H373: May cause damage to organs through prolonged or repeated exposure (kidney))
Molybdic acid (7782-91-4)	-
Tetraammonium hexamolybdate (12411-64-2)	-

1

3 Manufacture and uses

3.1 Manufacture

Molybdenum processing is a multi-stage operation involving the extraction and refinement of molybdenum from molybdenite ore, a mineral of molybdenum disulfide.[15] This process begins with mining, where the ore is extracted from underground or open-pit mines. The mined ore is reduced in size by milling it through crushing and grinding. Subsequently, the ore undergoes a flotation process, in which it is mixed with liquid and air. This flotation step effectively separates molybdenum sulfide from other minerals. Acid leaching can remove impurities such as copper and lead. Following flotation, the molybdenum concentrate is subjected to high temperatures during the roasting procedure, which transforms molybdenum sulfide into molybdenum trioxide. The molybdenum trioxide is then chemically converted through a series of hydrogen reductions into pure molybdenum products.[15]

3.2 Identified uses

Molybdenum is a naturally existing trace element widely distributed in nature. It serves an essential biological function as a micronutrient in various organisms, including plants, animals, and humans.[2]

For the general population, the main way of being exposed to molybdenum is through the consumption of food. Other potential exposure routes like breathing in ambient air, drinking water, or skin contact are not significant for most people. However, in certain work environments these alternate routes of exposure could be more relevant.[2] Molybdenum is primarily used in metallurgical applications, including as an alloying agent in cast iron, steel, and superalloys to enhance properties such as hardenability, strength, toughness, and wear- and corrosion-resistance. Molybdenum is commonly used in combination with other alloy metals like chromium, cobalt, manganese, nickel, niobium, and tungsten. The leading form of molybdenum used by industry, particularly in stainless steel production, is molybdenum trioxide.[2]

Molybdenum is also used significantly as a refractory metal and molybdenum compounds in a variety of non-metallurgical chemical applications, such as catalysts, lubricants, and pigments.[2]

Molybdenum and its compounds are registered under REACH for use as intermediate in manufacturing other chemicals and for the manufacturing of amongst others paper products, plastic products, machinery and vehicles, fabricated metal products, and electrical, electronic and optical equipment. Industrial products include water treatment chemicals, anti-freeze products, metal working fluids, washing & cleaning products, biocides (e.g. disinfectants, pest control products), fertilizers, pH regulators, lubricants and greases, and paper and chemicals dyes. Consumer products reported under REACH include anti-freeze products, heat transfer fluids, fertilizers, water treatment chemicals and adsorbents.[1]

4 Toxicokinetics

A detailed description of the toxicokinetics of molybdenum compounds in humans and animals was provided in an advisory report by the Health Council of the Netherlands from 2013 and presented below. Additional relevant information was added based on an RIVM report and the evaluation of the ATSDR.[2, 16]

4.1 Absorption

Several variables can influence how molybdenum is absorbed orally, with absorption rates ranging from 40% to 100%.[17, 18] Higher doses typically result in decreased absorption, especially when molybdenum is consumed with a meal.[17] While there is evidence suggesting absorption of airborne molybdenum, there is no quantitative data available of the exact amount absorbed.[2] Molybdenum exhibits poor absorption through the skin, estimated to be around 0.2%.[19] A brief summary is given below.

Inhalation

Molybdenum particles that are inhaled and settle in the respiratory tract undergo three main distribution processes: (1) they are transported by the bronchial and tracheal mucociliary system to the gastrointestinal tract; (2) they are transported to thoracic lymph nodes such as those in the lung, tracheobronchial area, and mediastinum; or (3) they are absorbed into the bloodstream and/or lymphatic system and then transferred to other tissues like peripheral lymph tissues, liver, and kidney. These processes are applicable to all types of deposited molybdenum, although the contributions and rates of each pathway vary depending on the physical characteristics such as particle size and solubility.[20, 21]

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.[22] This classification supports an expected terminal absorption half-time of around 19 years.[20, 21] Conversely, more soluble forms of molybdenum, such as molybdenum trioxide ($Mo^{VI}O_3$), are expected to dissolve and absorb more rapidly.[2]

No human data are available on inhalation exposure. In one animal study published in 1945, guinea pigs were used for a short-term inhalation study to test for tissue distribution and gross pathology.[23] The animals (24-25 animals per group) inhaled high amounts of dust containing molybdenum trioxide (average concentration of 205 mg molybdenum/m^3, corresponding to 310 mg molybdenum trioxide/m^3), molybdenite (molybdenum disulfide) (286 mg molybdenum/m^3, corresponding to 607 mg molybdenite/m^3), or calcium molybdate (159 mg molybdenum/m^3, corresponding to 388 mg calcium molybdate/m^3). Exposure was performed for one hour per day, five days per week for a total of five weeks. At the end of the exposure period half of the animals were killed for analysis of molybdenum content in various tissues organs (i.e., the liver, kidneys, lungs, spleen and bones). The other half of the animals were allowed to live for two weeks longer, with no molybdenum exposure, before they were also sacrificed. Data were compared with non-exposed controls. After exposure, molybdenum trioxide dust was found in all tissues examined (the highest amounts in the kidneys and bones). Calcium molybdate was mainly found in the lungs, the kidneys and bones. Molybdenite dust gave merely negative results (according to the authors, no data presented). The authors also reported on exposure to molybdenum sulphide. High levels of molybdenum sulphide were found in the lungs, but levels of molybdenum in the liver, kidneys, spleen and bones did not exceed the levels found in non-exposed animals. The authors considered molybdenum sulphide as a very insoluble compound, and molybdenum trioxide dust (and fume) as soluble. No quantitative data or further details were presented on how much of the molybdenum compounds were actually absorbed by the lungs.

Oral intake

Giussani et al. (2006) investigated the intestinal absorption of molybdenum in seven healthy volunteers by simultaneous oral administration (water, tea or composite meals), and intravenous injection, of stable isotopes of molybdenum.[17] For this, isotopic solutions were prepared using metal molybdenum powders enriched in ^{95}Mo and ^{96}Mo, respectively. Their results indicated that molybdenum ingested orally (in liquid form) was rapidly and totally absorbed into the circulation. The rate and extent of absorption depended on the composition of the meals. A comparable result was reported by Werner et al. (1998).[18] Turnlund et al. (1995) investigated molybdenum absorption, excretion and retention with stable isotopes, in four healthy volunteers.[24] They were given a low-molybdenum diet (22 µg/day) for 102 days, followed by the same diet supplemented with molybdenum (ammonium paramolybdate dissolved in deionized

water) to contain 467 µg/day for another eighteen days. The stable isotopes ^{100}Mo (prepared for diet), ^{97}Mo (prepared for intravenous injections) and ^{94}Mo (used as an isotopic diluents) were used as tracers. The isotopes were purchased as metal powders. The oral absorption of ^{100}Mo averaged 88% in the low-molybdenum diet, and 93% in the high-molybdenum diet. Turnlund also studied molybdenum kinetics after consumption. Using a comparable design as the previous study, and using compartmental kinetic models, it was estimated that the residence time for molybdenum in the gastro-intestinal tract was at 1.7 ± 0.4 days; in plasma molybdenum retention time averaged 22 ± 4 minutes, whereas slow-turn-over tissue (possibly hepatic) retention averaged 58 ± 16 days.[25] In various animal species (e.g., guinea pigs, rabbits) absorption of ingested soluble and insoluble molybdenum compounds was reported, the absorption being dependent on solubility and diet composition, and varying between 40 and 85%.[23, 26-28]

Dermal uptake

Sodium molybdate dihydrate was tested in vitro for dermal absorption using skin membranes according to OECD test guideline (TG) 428 (data obtained from the European Chemicals Agency: //echa.europa.eu/). Doses applied to the skin were 105 and 542 µg/cm^2. The percentage of the doses absorbed by the skin, including stratum corneum were 0.21 and 0.16% (after eight hours of exposure and 16 hours post-exposure monitoring). No other human or animal data available.

4.2 Distribution

In humans and various animal species, molybdenum is present in low concentrations in all the fluids and tissues in the body; in plasma, molybdenum is bound to α_2-macroglobulin in the form of molybdate.[29] The greatest amounts are found in the kidneys, liver, and the bones. Lower levels are found in the adrenal glands.[23, 26-28] Overall, substantial individual variation in the molybdenum blood level occurs, because plasma molybdenum reflects molybdenum intake by food and water products.[30] Average plasma concentrations range between 0.3 to 1.1 µg/L (3 to 11 nmol/L).[29-32] This level may increase up to 400 µg/L in persons near areas rich in molybdenum or near molybdenum mining centers.[32] There is no apparent bioaccumulation of molybdenum in human or animal tissue, and when exposure is withdrawn, the tissue concentrations quickly return to normal.[32]

Maternal-Foetal Transfer

Studies in both humans and animals have demonstrated that molybdenum is transferred to the foetus. Specifically, one study involving 33 maternal-foetal pairs at childbirth revealed comparable levels of molybdenum in both maternal and foetal cord blood samples, with averages of 1.44±0.75 µg/L for maternal blood and 1.44±0.89 µg/L for foetal blood.[33] Additionally, research showed that molybdenum concentrations in venous cord blood, which flows from the placenta to the foetus, were slightly higher (0.7±0.2 µg/L) than in arterial cord blood, which flows from the foetus to the placenta (0.6±0.2 µg/L). This suggests that molybdenum is retained in the foetus.[34] However, the study did not analyse whether there was a statistically significant difference between the molybdenum concentrations in venous and arterial blood.

Maternal-Infant Transfer

Several studies have investigated the levels of molybdenum in breast milk.[35-43] These studies have reported mean concentrations ranging from 0.02 to 72 µg/L. Molybdenum levels in breast milk are typically highest at the beginning of breastfeeding and then decrease over time.[44] However, in the sole study that compared maternal intake to molybdenum levels in breast milk, conducted by Wappelhorst et al. (2002), no correlation was found between maternal molybdenum intake and concentrations of molybdenum in breast milk.[43] In this study, the mean concentration of molybdenum in breast milk was reported as 72 µg/L, while the mean maternal intake was 132 µg/day.

4.3 Metabolism

Metals are not metabolised. However, molybdenum can exist in different forms and undergoes changes in its oxidation state. Under physiological conditions, molybdenum compounds solely exist in the form of molybdate (see Section 2.2).

4.4 Elimination

Under normal exposure conditions, molybdenum intake and excretion are balanced in humans and animals. In humans, absorbed molybdenum is excreted through both urine and faeces, with urine being the dominant excretion route, responsible for excreting approximately 75–90% of the absorbed dose.[45, 46] The excretion is rapid, and

is enhanced by the presence of high dietary levels of copper and sulphate.[31, 32] The fraction excreted in urine also increases with increasing dietary intake.[46] In a twelve day period, 20% of the dose of molybdenum fed during intake of a low-molybdenum diet (in the form of ^{100}Mo) was excreted in the urine, 12% was excreted in the faeces, and 68% remained in the body.[24] The excretion percentages of ^{100}Mo in the group fed a high-molybdenum diet, were 71% (in the urine) and 7.3% (in the faeces); the percentage retention in the body was 21%. In animals percentages of urinary excretion of between 36 and 90% have been reported.[23, 32] Furthermore, kinetic modelling suggested that low intake resulted in adaptation to conserve body molybdenum, whereas high intake results in increased elimination of molybdenum.[46, 47] The blood half-life for molybdenum may vary from several hours in laboratory animals up to several weeks in humans.[29, 32]

5 Mechanism of action and toxicity

Molybdenum can exist in different forms and undergoes changes in its oxidation state. The primary form that interacts with enzymes is Mo^{VI}.[48] When molybdate is taken into a cell, it combines with molybdopterin to create molybdenum cofactor (Moco), which is a sulfur-molybdate complex and an essential component of molybdenum-dependent enzymes.[49, 50] It is believed that Moco is bound to a Moco-binding protein within the cell, as it is extremely sensitive for oxidation.[49] In this storage form, however, Moco will be readily available to meet the cell's need for molybdenum enzymes. Additionally, molybdate can form complexes with copper and attach to plasma proteins as a copper-molybdenum-sulfur complex.[51, 52]

The precise mechanism underlying molybdenum toxicity remains unclear. There are indications that altered copper utilization is a significant factor, as demonstrated by studies that show more severe effects in copper-deficient animals. Studies show that molybdenum increases copper levels in the plasma, liver, and kidneys. Notably, the adverse effects can be reversed with high copper doses.[2] The observed effects in animals exposed to molybdenum, such as decreased body weight and anaemia, resemble those in copper-deficient animals.[53-57]

In ruminants, it appears that molybdenum reacts with sulfate in the rumen to form thiomolybdates, which can bind to copper and hinder its absorption, leading to functional copper deficiency.[58] In monogastric animals, such as rats, exposure to sodium molybdate shows toxicity, which can be mitigated by administering sulfate.[59, 60] However, when rats are fed diets containing molybdate and sulfide, it results in increased plasma molybdenum and copper levels, reducing ceruloplasmin activity.[61] When rats were exposed to tetrathiomolybdates, similar effects were seen as observed in ruminants with signs of copper deficiency.[62] Some molybdenum compounds, as observed in studies in young men, can increase serum and urine copper levels, while another study showed no significant alterations in serum copper levels in humans exposed to various molybdenum levels.[63, 64]

Several studies have reported that molybdenum can induce oxidative stress. An in vitro study using mouse fibroblasts and liver cancer cells demonstrated that trivalent molybdenum induced oxidative stress, which was demonstrated by increased reactive oxygen species generation and a higher malondialdehyde concentration.[65] This mechanism is supported by observational studies, such as a general population study

demonstrating an association between urinary molybdenum levels and the ratio of oxidized glutathione to reduced glutathione, indicating a potential association between molybdenum and oxidative stress.[66] Additionally, research in mice showed that molybdenum-induced sperm effects were correlated with levels of enzymatic antioxidants. Lower molybdenum doses enhanced antioxidant levels and improved sperm parameters, while higher doses led to decreased antioxidant levels and sperm abnormalities.[67] Similar findings for superoxide dismutase and glutathione peroxidase levels were reported in the ovaries of mice and the rate of abnormalities in MII oocytes.[68]

6 Adverse effects on sexual function and fertility

6.1 Human data

An overview of the epidemiological studies on adverse effects on sexual function and fertility is provided in Table A1-2 of annex A. These studies include cross-sectional studies only.

Male fertility

In 2008, Meeker et al. reported on semen quality (sperm count, sperm concentration, percent motile sperm, and sperm morphology), and metals in blood among men recruited through fertility clinics after molybdenum exposure (N=219).[69] They found decreases in sperm concentration and abnormal morphology, when adjusted for age, current smoking, and the impact of multiple metals on semen quality simultaneously (odds ratios (OR) for sperm concentration: metal percentile 70-85th(corresponding to 1.0 µg/L - 1.5 µg/L of molybdenum in blood), 2.23 (95% confidence interval (CI), 0.67-7.60); metal percentile >85th, 6.26 (95% CI, 1.57-25.0). OR for sperm morphology: metal percentile 70-85th, 0.91 (95% CI, 0.37-2.24); metal percentile >85th, 3.44 (95% CI, 1.23-9.67)).[69]

In 2010, Meeker et al. reported on reproductive hormone levels (serum FSH, LH, inhibin B, testosterone, and SHBG) among the same group of men.[70] The authors found a significant inverse trend between molybdenum concentrations in blood and testosterone levels, also when correcting for exposure to other metals (Regression coefficient (95% CI): metal percentile 70th –85th (corresponding to 1.0 µg/L – 1.5 µg/L of molybdenum in blood): −18.5 (95% CI −53.3, 16.3), metal percentile > 85th: −55.9 (95% CI −92.5, −19.3), p for trend = 0.003 .[70]

Guzikowski et al. (2015) studied associations between molybdenum and other metal concentrations in semen and sperm count, motility, and morphology in 34 men (aged 26-42 years) from primary infertile couples in the rural area of Opole, Poland.[71] Of these, 23 men (68%) had at least one sperm quality parameter below the reference value (sperm concentration <20×10^{6}/ml, <50% motile sperm, and/or <15% normal

forms). No correlations (Pearson's r, alpha = 0.05) were found between molybdenum concentration and the three sperm quality parameters.[71] The absolute concentrations of the metals in semen were not provided. The committee noted that the results were not adjusted for other covariates and the study population was relatively small.

Lewis & Meeker (2015) studied the associations between urinary molybdenum (and other metals) and serum testosterone in 484 men aged 18-55 years from the NHANES general population cohort in the USA in 2011-2012.[72] The geometric mean of the urinary molybdenum concentration was 41.54 µg/L (10^{th} percentile 11.80 µg/L, 95^{th} percentile 141 µg/L). An inverse association was found between urinary molybdenum concentration and testosterone (-4.26% (95% CI: -7.7 - -0.69) when adjusted for age, BMI, income, race, serum cotinine, and urinary creatinine.[72] A substantial portion of the original NHANES study population was excluded from the analysis. Additionally, absolute values and distribution on serum testosterone levels were not provided.

Skalnaya et al. (2015) studied the correlations between the concentrations of molybdenum (among other metals) in semen and with sperm quality in 148 volunteers in Orenburg, Russia.[73] The authors reported an inverse association (Spearman's r, $p<0.05$) between molybdenum concentration (concentrations were not included in the publication) and seminal liquid volume, whereas no associations were found with sperm count, sperm concentration, sperm motility, and sperm vitality.[73] The committee noted that the method did not clearly specify which outcome measures were included, that certain tables were missing, and results were not adjusted for confounding factors.

Zeng et al. (2015) studied the associations between urinary level of molybdenum (among other metals) and below-reference semen quality parameters in 394 men presenting for semen analysis at a reproductive centre in Wuhan, China.[74] The geometric mean of the urinary molybdenum concentrations was 44.45 µg/g creatinine (25th percentile 28.99 µg/g creatinine, 75th percentile 68.46 µg/g creatinine). No associations (p for trend >0.05) were found between quartiles of molybdenum spot urine concentration and below-reference sperm concentration (n=46), sperm motility (n=222), sperm count (n=38), and sperm morphology in multivariable logistic regression analyses adjusted for age, abstinence time, and smoking status. Molybdenum was not retained in analytical models including multiple metals.[74]

Wang et al. (2016) studied associations between urinary level of molybdenum (among other metals) and markers of male reproductive health in 1052 men of subfertile

couples in Wuhan, China.[75] Outcomes included spermatozoa apoptosis (n=460), sperm DNA-damage (n=516) and sex hormones in serum (n=511). Quartiles of average geometric mean molybdenum concentration (µg/L) from two repeated urine samples were analysed in multivariable linear regression models, adjusted for age, BMI, smoking, and urinary creatinine and false-discovery rate. The geometric mean of the first sample was 67 µg/L (interquartile range 44-106 µg/L) and of the second sample was 57 µg/L (interquartile range 36-103 µg/L). A lower total testosterone/luteinizing hormone ratio was associated with a higher level of molybdenum (p for trend 0.02). When also adjusting for other urinary metal levels, this ratio was 5.6%, 8.9%, and 16% lower for the 2nd, 3rd, and 4th quartiles of molybdenum concentration, respectively, compared to the first quartile (p for trend 0.03). No associations were found between molybdenum concentration and apoptosis markers or sperm DNA integrity markers.[75]

Zhou et al. (2016) studied associations between urinary levels of molybdenum (among other metals) and sperm DNA damage in 207 men from subfertile couples in Wuhan, China.[76] The geometric mean of urinary concentrations of molybdenum was 39.34 µg/g creatinine (interquartile range 26.77 – 58.60 µg/g creatinine). No associations were observed between quartiles of molybdenum spot urine concentration and comet assay parameters (%DNA tail, tail length, and tail distributed moment) in multivariable models adjusting for age, BMI, smoking status, and abstinence time.[76] The committee noted that the study population partially overlaps with the study by Zeng et al (2015).

Branch et al. (2021) assessed associations between concentrations of molybdenum (among other metal(loid)s) in urine and 7 measures of semen quality among 413 reproductive-aged men from the LIFE-study recruited from 16 US counties between 2005–2009.[77] Semen quality endpoints were total sperm count, semen volume, sperm concentration, next day motility, traditional morphology, % DNA fragmentation index, and % high DNA stainability. The urinary molybdenum concentration in this general population sample was relatively low (median 47 µg/L). In multivariable linear regression models for molybdenum concentration without taking into account other metal(loid)s, no associations with semen quality endpoints were observed when adjusting for lifestyle and other potential confounders. Taking into account all 15 metal(loid)s under study, penalized LASSO regression models were fitted to identify and select metal(loid)s most likely to be predictive of each semen quality endpoint. Molybdenum concentration was only selected for inclusion in a subsequent multi-metal(loid) and confounder-adjusted linear regression analysis on sperm motility, resulting in a statistically non-significant beta coefficient of 0.07 (95% CI: -0.3-0.44).[77]

Female fertility

Syrkasheva et al. (2021) studied associations between the concentrations of molybdenum (among other metals) in blood and health outcome parameters related to assisted reproductive technologies (ART) treatment in 30 subfertile women in Moscow, Russia.[78] The median blood molybdenum concentration was 0.705 µg/L (interquartile range 0.640-0.860 µg/L). Associations between blood molybdenum concentration and reproductive outcomes were evaluated by calculating Pearson's correlation coefficients and using Mann-Whitney U or chi-square tests, without adjustment for other factors. No associations were found between concentrations of molybdenum and outcome parameters considered (including levels of β-human chorionic gonadotropin (β-hCG), anti-müllerian hormone (AMH), and free thyroxine (T4) in blood, clinical pregnancy, number of previous pregnancies, gynaecological diseases, primary or secondary infertility, features of the ovarian stimulation protocol, and parameters of oogenesis and early embryogenesis).[78] It should be noted that in this study, the outcome measures may precede exposure hampering a causal interpretation or were linked to the treatment received.

6.2 Animal data

An overview of the *in vivo* studies on adverse effects on sexual function and fertility is provided in Table A3 and A4 of annex A.

Jeter et al. (1954) administered doses of <1, 20, 80, or 140 ppm molybdenum (approximately <0.04, 0.9, 3.5, 6.2 mg molybdenum/kg bw/day) as disodium molybdate dihydrate in diets containing 5 ppm copper (normal copper content 1.8 ppm) to Long-Evans rats (N=4 or 8/sex/group) for about 20 weeks.[79] Depigmentation of the hair and alopecia were observed in some rats fed 20, 80 or 140 ppm dose groups. The average weight gain of male rats was statistically significantly lower in the 20, 80 or 140 ppm dose groups, as well as in the females dosed 80 and 140 ppm. The average weight gain over the first eleven weeks was decreased (controls: 176 g (males) 128 g (females), 80 ppm: 147 g (males) 105 g (females), 140 ppm: 80 g (males) 85 g (females). Animals were allowed to mate from eleven weeks onwards. At 80 and 140 ppm molybdenum, males were successful in mating in one of four cases. Mating of the treated males with untreated females did not result in pregnancy. Mating of females given 80 or 140 ppm molybdenum with untreated males resulted in pregnancy rates of

100%. Histopathologic examination of the testes of infertile males treated with 80 and 140 ppm molybdenum revealed degeneration of the seminiferous tubules.
In order to determine the effect of molybdenum upon oestrus cycle, 4 females were fed a ration containing 700 ppm of molybdenum and vaginal smears were made over a 5-week period. Mature virgin female rats showed irregular oestrus cycles after receiving the rations containing 700 ppm molybdenum for 10 days, whereas controls had a normal oestrus cycle.[79] The committee considers the number of animals too low to draw such a conclusion upon the oestrus cycle.

Schroeder et al. (1971) exposed five pairs of Charles River CD mice to 10 mg/L molybdenum (as molybdate; cation unknown) in deionized drinking water for up to six months, while the diet contained 0.45 ppm molybdenum.[80] (Assuming a mean water intake of 167 to 200 mL/kg bw/day and a food intake of 120 to 150 g/kg bw/day, the total intake of molybdenum per day approximates 1.7 to 2 mg/kg bw) Animals were allowed to breed freely during this period. Animals were at random selected from the first three litters to form the F1, and allowed to breed to form the F2 (period not indicated). Animals of the first two F2 litters were selected to form the F3-generation. No mortality was observed in the F0-generation. Molybdenum did not affect the growth rate in the F0-generation. Age at first litter and interval between litters were similar to control values. No other data on this generation are available. In the F1-generation, no differences between treatment group and controls were reported for number of litters, litter size and number of runts. Fifteen of the 238 F1 offspring died early (not further specified, 0 in controls). In the selected breeding pairs of the F2-generation, one female died. In this generation, the interval between the litters was increased (43 versus 28 days in controls), but the age at first litter was not affected. The number of F2 litters and litter size, and young deaths were similar to controls. Five of the 26 litters were found dead compared to 0 out of 23 in controls. In the selected F3 pairs, four female deaths were reported, and the age at first litter was increased from 62 to 79 days. No effect on interval between litters was found. The number of litters and litter size were decreased in treated animals. Four litters in the F3 were found dead versus zero in controls. The numbers of runts (11 versus 0 in controls) and dead young (34 versus 1 in controls) were increased. Furthermore, the experiment concerning the F3 generation was discontinued.[80] There are no further details on the selection of the litters. The number of pairs selected in the F1 and F2 was not reported. No details on the dead pups and their incidence along different litters was reported for any of the generations. 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.

Fungwe et al. studied weanling female Sprague-Dawley rats (N=21/group) that were given drinking water with 0, 5, 10, 50 and 100 mg/L molybdenum as sodium molybdate dihydrate for 6 weeks.[81] (Assuming a mean water intake of 50 to 125 mL/kg bw/day for SD rats, the units in mg/L correspond to a daily intake of approximately 0.25-0.625 mg/kg bw (5 mg/L), 0.5-1.25 mg/kg bw (10 mg/L), 2.5-6.25 mg/kg bw (50 mg/L), and 5.0-12.5 mg/kg bw (100 mg/L).) Thereafter, rats were exposed during three oestrus cycles before being mated with untreated males (N=15/group) or sacrificed (N=6/group). The mated females remained exposed during gestation until necropsy on day 21. During the first six weeks of the study, no effects on body weight became apparent. 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. Pregnancy rate was not affected by treatment.[81]

Howell et al. (1993) studied the effect on the trace element status, and reproductive capacity of guinea pigs of ammonium molybdate (AM) and thiomolybdate (TM, presumably ammonium tetrathiomolybdate) in drinking water.[82] Mature female (N=8/dose) and male (untreated; 12 in total) Hartley albino guinea pigs, weighing around 500-600 g, were fed ad libitum a diet containing 212 µmol copper/kg diet. When each female entered the third oestrus cycle, males were introduced twice a day. Females of dose groups A (control), B (261 µmol AM/L), C (261 µmol TM/L), and D (130 µmol TM/L) received molybdenum compounds from the first day of the oestrus cycle onwards, whereas treatment of group E (261 µmol TM/L) and F (130 µmol TM/L) females was started immediately after mating. (Based on Mol Wt. of AM divided by atomic mass of Mo, (B) 8.70 mg AM/kg bw is equivalent to 4.71 mg Mo/kg bw. Based on Mol Wt. of TM divided by atomic mass of Mo, (C, E) 11.55 mg TM/kg bw is equivalent to 4.26 mg Mo/kg bw, (D, F) 5.75 mg TM/kg bw is equivalent to 2.12 mg Mo/kg bw) Subcutaneous oedema was found only in 1/8 and 4/8 female adult guinea pigs of the high TM dose groups, C and E. All adult females had oestrus cycles and conception rates that were reported to be unaffected. The number of pregnant animals was: group A 7, group B 4, group C 6, group D 6, group E 8, group F 6. The number of surviving pregnant females was: group A 7, group B 4, group C 3, group D 4, group E 0, group F 6.[82]

A dose-range finding study was performed in which Sprague-Dawley rats (10/sex/dose group) received sodium molybdate dihydrate at 0, 3, 20 or 40 mg molybdenum/kg bw/day via drinking water or via diet.[83] Sperm parameters, litter observations, postmortem examinations of parental animals and offspring, reproductive indices and offspring viability indices were examined. The study included three dose levels via drinking water and three dose levels via the diet; Test item related reduction of the absolute body weight and of the body weight gain was observed in the males of the 40 mg molybdenum /kg bw/day exposure group via drinking water, and at the same dose level in the males and females exposed via diet. A dose related increase in molybdenum levels was observed in serum, liver and kidney of parental animals and in pups (no quantitative data available). This indicated absorption of molybdenum from both diet and drinking water with levels generally higher from diet than from drinking water in both parents and pups. Pregnancy rate was reduced (6 pregnant rats out of 10 vs. pregnancy rate controls 10 out of 10) in the 40 molybdenum/kg bw/day drinking water exposure group and was outside the historical control average. A reduction in the number of live born pups with an increase in stillborn pups was observed upon treatment via drinking water. The stillborn pups were all in a single litter and the overall mean litter size did not differ between groups. No treatment-related effects were observed in any of the other parameters.[83] Based on the results of this range-finder study, three doses up to 40 mg molybdenum /kg bw/day via drinking water were selected by the study authors and one dose of 40 mg molybdenum /kg bw/day via diet for the evaluation in the full two-generation study as published by Murray et al. (2019).

In a two-generation study, performed according to OECD TG 416, groups of 24 male and 24 female Sprague-Dawley rats were administered sodium molybdate dihydrate at 0, 5, 17, or 40 mg molybdenum /kg bw/day in the drinking water or 40 mg molybdenum/kg bw/day in the diet over two generations to assess reproductive toxicity.[84] A statistically significant increase in the average number of primordial follicles was observed in the left ovary of parental females at 17 mg/kg bw/day (drinking water) and 40 mg/kg bw/day (diet). This was also observed in the F1 generation in the right, left, and combined ovaries at 17 and 40 mg/kg bw/day (drinking water) and 40 mg/kg bw/day (diet). All values were within the historical control range. The percent of sperm with no head was statistically significantly increased in the parental generation given 40 mg/kg bw/day in the diet compared to the control value. A slight increase (not statistically significant) was observed in the percent of no head sperm in the group given 40 mg/kg bw/day in drinking water. In both cases, they were largely attributable to one male in each dose group. Average values were within historical control range.

No other adverse effect on reproductive function was observed at any dose level in either generation as indicated by no significant dose-related effect on oestrus cycles, sperm parameters, mating, fertility, gestation, litter size, pup survival or growth. Systemic toxicity, including decreased body weight, food consumption (males only) and water consumption, was observed among both sexes given 40 mg Mo/kg bw/day in the diet. Serum levels of molybdenum and copper were increased in a dose-related manner.[84] The committee considered the applied doses too low for evaluation for adverse effects on fertility.

In another study by Murray *et al.* (2023) Sprague-Dawley rats were administered sodium molybdate dihydrate in drinking water.[85] This study aimed to repeat and confirm the findings that were previously described by Fungwe et al. (1990) for both development and reproductive toxicity. The chosen dose levels of 0, 20 or 40 mg molybdenum/ kg bw/day differed from those chosen by Fungwe et al and were based on the NOAEL that the authors deduced in 2014 in a developmental toxicity study (see section 7.2). Because of the hypothesis that the difference in copper diets caused the differences in findings between Fungwe et al. and the guideline studies, the copper concentration was accommodated to a concentration of 6.2 ppm in the rats' diet, which is similar to the concentration that Fungwe et al used. Although the authors aimed to replicate the study, the authors describe some differences in the experimental design between the two studies related to the dose levels, group sizes, and exposure duration. Murray et al did not find sodium molybdate dihydrate related effects on mating or fertility parameters. 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).[85]

Effects on reproductive organs from repeated dose studies

In a NTP-study (1997), Fischer 344 rats (N=10/sex/dose) and B6C3F1 mice (10/sex/dose) were exposed to 0, 10, 30, and 100 mg molybdenum trioxide/m^3 (in aerosol) by inhalation, for 6.5 hour per day, 5 days per week for thirteen weeks.[86] Body and organ weights, clinical chemistry and haematological parameters, and histopathological findings were not different from the control values. In exposed male rats, sperm counts were unaffected. In addition, no statistically significant effect was observed on the concentration of epididymal spermatozoa. At 10, 30 and 100 mg/m^3, rats showed slightly decreased absolute epididymis weights (0.48 g, 0.49 g and 0.47 g, respectively) compared to unexposed rats (0.50 g). However, these effects were not statistically significant. In exposed mice, absolute cauda epididymis weight was slightly

increased (0.025 g *versus* 0.018 g in controls) at 10 mg/m^3, and absolute testis weight was slightly decreased (0.10 g *versus* 0.12 g in controls) at 100 mg/m^3. However, these effects were not statistically significant. 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 also performed a long-term carcinogenicity study, in which rats and mice were exposed to the same molybdenum trioxide levels as in the thirteen-week study (for details on study design see Section 7.2.4). Examination included the occurrence of non-neoplastic lesions. No lesions were found in the genital system of males and females that could be related to exposure to molybdenum.[86] The NTP did not specifically examine sperm pathology.

Pandey and Singh (2002) administered 0, 10, 30, or 50 mg sodium molybdate per kg bw by gavage, 5 days/week for 60 days to groups of 10 adult male Druckery rats (body weight at start of experiment averaged 120 g).[87] (Based on Mol Wt. of sodium molybdate divided by atomic mass of molybdenum, 0, 10, 30, and 50 mg sodium molybdate/kg bw is equivalent to 0, 4.7, 14.0, 23.3 mg molybdenum/kg bw) Body weights were measured at the start and end of the experiment and the rats were sacrificed in order to evaluate organ weights of the testes, epididymis, seminal vesicles and prostate glands. Molybdenum content (in only the highest dose group) was determined in the testis, epididymis, and seminal vesicle and these tissues were used for histopathological and biochemical assessment (for testicular enzymes sorbitol dehydrogenase, lactate dehydrogenase and g-glutamyl transpeptidase). Spermatozoa were counted and sperm motility and morphology were assessed. No effects on body weight or clinical signs that could be related to treatment were observed. 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 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). The authors derived a NOAEL of 10 mg sodium molybdate/kg bw from this study.[87]

The International Molybdenum Association (IMOA) commissioned two separate animal experiments, in which Sprague-Dawley CD rats were given sodium molybdate

dihydrate by gavage or via the diet.[88, 89] In one experiment, the animals (5 animals/sex/group) were given the compound by gavage (once daily) or in their diet (*ad libitum*), for 28 consecutive days.[88] Doses administered were 0, 4 or 20 mg molybdenum/kg bw/day. Also, another group of animals received the compound by gavage twice daily (10 mg/kg bw/administration for a total of 20 mg/kg bw/day). At the end of the treatment, all animals were killed and postmortem examinations, including microscopic pathology, were performed. Analysis of blood samples revealed that molybdenum was present in the system. The investigators did not find exposure-related adverse effects on any in-life parameters (survival, body and organ weights, food consumption).[88]

In a 90-day study, Sprague-Dawley CD rats (10 or 20 animals/sex/group) received sodium molybdenum dihydrate at doses of 0, 5, 17, and 60 mg molybdenum/kg bw/day, for 91 or 92 days in their diet.[89, 90] At the end of the treatment ten animals of each group were killed for postmortem examinations. The remaining ten animals (in groups administered 0 or 60 mg molybdenum/kg bw/day) were allowed to recover for a further 60 days, before they were also killed for postmortem examinations. The study complied with OECD TG 408, with additional examination of oestrus cycles and sperm count, motility, and morphology. In males and females, the mean body weight changes from baseline were statistically significantly lower at the highest dose level. Furthermore, a statistically significant lower absolute body weight of 15% was observed among male animals from the highest dosed group. These reductions were partially explained by lower food intake. Furthermore, microscopic examinations revealed slight diffuse hyperplasia of the proximal tubules in the kidneys of two female rats fed 60 mg molybdenum/kg bw/day. No adverse effects were observed in the high dose animals after the 60-day recovery period, with the exception that male rats did not fully recover from reduced body weight. No treatment-related adverse effects on reproductive organ weights or histopathology, oestrus cycles or sperm parameters were observed at any dose level. A slight, but statistically significant decrease in progressively motile sperm was observed at 60 mg molybdenum/kg bw/day in males at the terminal sacrifice (59.0% versus 69.4% in the control group). The authors of the study suggested that this effect could be attributed to the control group, which had a value that approached the upper end of the historical control range for this parameter (mean of 59.8% ± 16.2%).[89, 90]

The effects on sperm parameters and testicular oxidative stress were investigated in a sub-acute toxicity study.[91] ICR mice were exposed to 0, 12.5, 25, 50, 100 or 200 mg/L sodium molybdate dihydrate in drinking water for 14 days (corresponding to 0, 2.5, 5,

10, 20 and 40 mg/kg bw/d based on ECHA guidance R.8). 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 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.[91]

A 14-day toxicity test was performed to investigate the effects of sodium molybdate dihydrate on ovarian parameters.[92] ICR adult female mice were exposed by free access to distilled water containing the sodium molybdate dihydrate at 0, 5, 10, 20, and 40 mg/L (corresponding to 0, 1, 2, 4 and 8 mg/kg bw/day) for 14 days. (Based on Mol Wt. of sodium molybdate dihydrate divided by atomic mass of molybdenum, 0, 1, 2, 4, and 8 mg sodium molybdate dihydrate/kg bw is equivalent to 0, 0.4, 0.8, 1.6, 3.2 mg molybdenum/kg bw.) 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.[92] The committee noted that the dose levels are relatively low.

Testicular tissues of molybdenum treated male mice were evaluated for oxidative and histopathological changes in relation to interference with copper (Cu) intake.[93] The mice were administered with high molybdenum (400 mg/ L drinking water, corresponding to 80 mg/kg bw/day based on the ECHA guidance R.8) and/or low copper concentrations in drinking water. 80 mice were equally divided in the control group (with 3 mg/L Cu), the high molybdenum group (with 3 mg/L Cu), the low copper group and the group with combined high molybdenum and low copper. 10 mice per group were anesthetized on the 50th day of administration and 10 mice per group on the 100th day of administration. Antioxidant status was analysed in blood serum and homogenized testes, morphological testicular changes were evaluated and epididymal sperm characteristics were assessed. 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 membrane breakage or disappearance. The mitochondria of spermatogenic cells and 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.[93] The committee noted that the authors did not specify which molybdenum compound was used. Additionally, the figure descriptions were inconsistent with the provided information on the y-axis in figures 2 and 3. No absolute data were presented for teratosperm as the rate was expressed in percentage. It remains unclear how much of which impacted sperm characteristics attributed to the teratosperm. Furthermore, it is unclear to the committee what the authors mean with the term “spermatogenic cells” and to which cellular developmental stage(s) towards mature sperm cell formation the authors refer. Also, the number of cells that were analysed is unclear.

The effect of sodium molybdate on testicular toxicity was investigated in adult Wistar rats.[94] Rats were treated by oral gavage with 0.05, 0.1, 0.2, and 0.4 mg/kg bw/day sodium molybdate (6 animals per dose group). (Based on Mol Wt. of sodium molybdate divided by atomic mass of molybdenum, 0, 0.05, 0.1, 0.2, and 0.4 mg sodium molybdate/kg bw is equivalent to 0, 0.02, 0.05, 0.09, and 0.19 mg molybdenum/kg bw) Control animals received distilled water. Following 30 days of administration, animals were sacrificed for biochemical and histopathological assays. 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.[94] The committee considered the doses as applied as too low to observe relevant effects.

6.3 Evaluation of the data

Epidemiological studies

There were nine cross-sectional studies that assessed the effects of molybdenum on male fertility. Two studies reported reduced sperm concentrations, sperm morphology, or serum testosterone levels in men exposed to molybdenum.[69, 70]

One study found an inverse association between urinary molybdenum concentration and serum testosterone.[72] It should be noted that this study may be susceptible to selection bias, and distribution and other descriptive results on serum testosterone levels were not provided. Another study that investigated the association of molybdenum and markers of male reproductive health observed an inverse association between molybdenum and the total testosterone/luteinizing hormone ratio.[75] Furthermore, no associations were found between molybdenum and the apoptosis markers or sperm DNA integrity markers. The study from Zhou et al. (2016) also assessed the effects of molybdenum on sperm DNA-damage, but found no associations between molybdenum concentrations and comet assay parameters.[76]

Four studies investigated the effect of molybdenum on semen quality. One study of limited quality found an inverse association, of unknown magnitude, between molybdenum concentration and seminal liquid volume, but found no effects on other sperm quality parameters.[73] However, this analysis was not adjusted for potential confounders and the study was reported poorly. Three other studies showed no effects of molybdenum on semen quality parameters.[71, 74, 77] It should be noted, however, that one study was considered of limited quality, since confounding could not be excluded as no other factors were considered in the crude analyses.[71] Additionally, the statistical analysis and reporting of this study was poor.

There was one study that investigated the effects of molybdenum on female fertility. However, no associations between molybdenum concentration and any of the health outcome parameters were found.[78] It should be noted that the study was reported poorly and a highly selective study population was used. Additionally, the outcome measures preceded exposure assessment or were linked to the treatment received, and no conclusions on the effect of molybdenum could be drawn.

Animal data

A two generation guideline study, including an accessory dose-range finding study, was performed with sodium molybdate dihydrate as the test substance in rats.[83, 84] In the dose-range finding study, effects on fertility were observed consisting of a reduced pregnancy rate in rats exposed to 40 mg/kg bw/day in drinking water.[83] These effects were not observed in the full multigeneration study with exposure via diet or drinking water. Nonetheless, Murray et al. (2019) did find effects within female ovaries consisting of an increase in the average number of primordial follicles in the parental and F1 generation at dose levels of 17 and 40 mg/kg bw/day.[84] Moreover, effects were found on sperm morphology as the percentage of sperm cells with no head was statistically significantly increased in the parental generation given 40 mg/kg bw/day molybdate dihydrate in the diet. In addition, a slight increase (not statistically significant) was observed in the percentage sperm cells with no head in the group given 40 mg/kg bw/day in drinking water.[84] Noteworthy, in both cases, the observed effects on sperm morphology were attributable to only a single male animal and average values were within the historical control range.

One poorly reported study observed effects of molybdates on male reproductive organs and sperm in rats,[87] and one study found a degeneration of the seminiferous tubules in rats.[79] In addition, the study by Jeter showed adverse effects on oestrus cycle,[79] but was poorly reported. Furthermore, there were indications that exposure to molybdates might affect female fertility in rats (prolonged oestrus cycle) in the study of Fungwe et al. (1990).[81] However, these effects were not confirmed by Howell et al (1993) and IMOA (2011).[82, 95] Furthermore, the study by Murray et al. (2023) did not confirm a prolonged oestrous cycle at 1.5 mg/kg bw/day in rats.[85]

In the NTP inhalation study (1997) with molybdenum trioxide no significant adverse effects on the male reproduction system in rats and mice were seen after inhalation exposure for 13 weeks.[86] In the NTP carcinogenicity studies no adverse effects were found in the genital system of males and females.[86]

One non-guideline study investigated the effects of molybdenum on testicular tissue in mice.[93] Histopathological examination of testicular tissue showed slight histological alterations in animals treated with molybdenum. However, the data descriptions were unclear because of inconsistencies between the data and descriptions for the rate of teratosperm and sperm motility.

Two 14-day repeated dose studies with sodium molybdate dihydrate in drinking water studied the effects on the reproductive organs in male or female mice.[91, 92] In both studies, positive effects were observed in the lower doses (at 1 mg/kg bw/day in females because of improved MII oocyte morphology, ovulation and ovary index and at 5 mg/kg bw/day in males because of a decrease in sperm abnormality rate), while higher doses had negative effects (at 8 mg/kg bw/day in females because of adverse effects on MII oocyte morphology, ovulation and ovary index and at 20 and 40 mg/kg bw/day in males because of an increase in sperm abnormality rate).[91, 92]

Another study investigated the effects of sodium molybdate on reproductive organs in male rats.[94] This study did not find any effects, but this could be attributable to the low dose levels used (up to 0.4 mg/kg bw/day). Nonetheless, inconsistent effects on the reproductive organs at the lower dose levels were observed between the multigeneration guideline study in rats (Murray) and the 14-day toxicity studies in mice (Zhai and Zhang), although adverse effects were found at higher doses in all these studies.

6.4 Conclusion

Human data

It should be noted that exposure levels in all the epidemiological studies were low. The results of Meeker (2010) were in line with more recent human studies that also found an inverse association with testosterone levels.[70, 72, 75] However, the committee does not classify substances based on testosterone outcome only. On the contrary, the adverse effects on fertility in men as reported by Meeker (2008) were not confirmed by the more recent human studies. One study found an adverse effect for only one sperm parameter,[73] this study was poorly reported and the analysis was not adjusted for potential confounders. Three other studies performed on sperm quality parameters found no adverse effects.[71, 74, 77] The only study on female fertility did not show any adverse effects.[78]

Overall, the available human data provided no sufficient evidence for classification for adverse effects of molybdenum or molybdenum compounds on sexual function and fertility.

Animal data

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.[79, 81, 82, 84, 85, 89]

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. The committee noted that rats have a high initial sperm reserve as opposed to humans. To affect rat fertility sperm counts have to be reduced by 90%.[96] Human males have highly variable sperm concentrations and are more easily affected in its fertility potential. This makes it more difficult to exclude functional effects in humans based on sperm effects found in rats. It should be noted that there are limitations in reporting of some of the studies. Moreover, the dose levels used in the reproduction studies were considered to be low and the results at similar dose levels were contradictory. The committee therefore considers the available evidence insufficient for a classification in Category 1B and recommends classifying molybdenum in Category 2.

Conclusion

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)).

7 Adverse effects on development

7.1 Human data

An overview of the epidemiological studies on adverse effects on development is provided in Table A5-7 of annex A. These studies include prospective cohort studies, case-control studies, and cross-sectional studies.

Prospective cohort studies

Shirai et al. (2010) evaluated the associations between maternal exposure to 10 metals, including molybdenum, and birth weight, birth length, and head circumference in 78 pregnant women visiting the obstetrics outpatient clinic of a hospital in Tokyo, Japan, in the period 2007-2008.[97] Recruitment and inclusion and exclusion criteria were only described in a general manner. Metal concentrations were measured in single spot urine samples collected between 9-40 gestational weeks during regular maternal health check-ups. The geometric mean (SD) molybdenum concentration was 79.0 (1.72) µg/g creatinine. No correlations or associations were observed between urinary molybdenum concentrations and birthweight, birth length, and head circumference.[97] However, the committee noted that the effect estimates and actual statistical data were not presented or reported. Additionally, it is difficult to determine whether the sample collection fell within the etiologically relevant period and to quantify the exposure. Potential residual confounding by alcohol intake or nutrition cannot be excluded. Due to the small population size and significant variations in the timing of molybdenum concentration measurements in urine, drawing conclusions becomes challenging.

Vázquez-Salas et al. (2014) studied the associations between prenatal exposure to molybdenum and infant neurodevelopment during the first 30 months of life in a random subsample of 147 mother-child pairs who participated in a prospective cohort study in Morelos, Mexico.[98] The concentration of molybdenum was determined in urine samples during each trimester of pregnancy with median values ranging from 48.9 to 59.1 µg/g creatinine. The Bailey test was administered at 1, 3, 6, 12, 18, 24 and 30 months of age. In multivariable generalized mixed effect models, inverse associations were found between urinary molybdenum concentration in the third trimester and the psychomotor development index (PDI). A doubling of molybdenum concentration

during the third trimester of pregnancy was associated with a PDI score that was 0.57 (95% CI 0.1 to 1.1) lower, adjusted for prenatal dichlorodiphenyldichloroethylene (DDE) exposure, gestational age, parity, maternal age, education, and IQ, birthweight, type of birth, sex of child, breastfeeding, and quality of home environment. The results indicated that no participants exceeded the clinical thresholds for the PDI scale of the Bayley test. No associations were observed for molybdenum concentrations in the first and second trimester and with the mental development indices (MDI) of the Bailey's scale, and the results of the Bayley test for MDI were within the normal limits.[98] The committee observed that, despite adjustment for multiple factors (even though selection of confounders was suboptimal), the clinical relevance of the observed inverse association remains questionable and was considered to be marginal. It should also be noted that there were some limitations in the urine measurements.

Bloom et al. (2015) assessed the impact of preconceptional parental urinary molybdenum concentrations and perinatal outcomes in 235 couples with singleton pregnancies from the LIFE prospective cohort in Texas and Michigan, USA.[99, 100] Median molybdenum concentrations were 31.4 µg/L and 48.9 µg/L in maternal and paternal urine, respectively. Multivariable linear and log-binomial regression models included tertiles of molybdenum concentration of both mothers and fathers, and were adjusted for maternal age, the difference between maternal and paternal ages, maternal and paternal smoking, income, race, total serum lipids (as a proxy for persistent organic pollutants) and creatinine. No associations were found between maternal or paternal molybdenum concentration in urine and gestational age, birth weight, birth length, head circumference, ponderal index, and infant sex, although boys were found to have a reduced head circumference of -0.57 cm (95% CI -1.11, -0.03) associated with continuous paternal molybdenum concentration in a subanalysis.[99, 100] It should be noted that the molybdenum concentration in the samples was low relative to the U.S. population values for 2005–2010, and no correction for multiple testing was conducted in this analysis.

In a prospective cohort study in Mexico City, Ashrap et al. (2020) collected urine samples from 212 women during their third trimester of pregnancy and their sons (n=118) at age 8–14 years.[101] The prenatal mean urinary molybdenum concentration was 19.5 µg/L, and the boys' peripubertal mean concentration was 46.6 µg/L. Associations between urinary concentrations of molybdenum and sex hormones in early adolescence, and indicators of sexual maturation in early and late adolescence were studied. An interquartile range (IQR) increase in in utero molybdenum

concentrations was associated with 51% (95% CI 19.1-92.4) higher testosterone concentrations (119% (31.5-266) in 94 boys who were prepubertal. No associations were found with oestradiol, Inhibin B, SHBG, and DHEA-S. Peripubertal molybdenum concentrations were not associated with any of the measured hormone concentrations. No associations were observed between in utero or peripubertal molybdenum concentrations and (changes in) genital development, pubic hair development, and testicular volume either.[101] However, the committee noted that it is difficult to detect and interpret such associations due to the small sample size and an insufficient follow-up time for 94/188 prepubertal boys.

In a prospective cohort study, Howe et al. (2020) investigated whether prenatal exposure to mixtures of heavy metals, including molybdenum, were associated with lower birth weight for gestational age in a predominantly lower-income Hispanic pregnancy cohort in Los Angeles (US).[102] Ten metals were measured in spot urine samples of 262 pregnant women participating in the MADRES cohort. Urine samples were collected in the first half of pregnancy (median gestational age 13.1 weeks). The median (IQR) urinary molybdenum concentration was 56.8 (42.9-80.7) µg/L. All analyses used a combination of metals, in which the contribution of molybdenum was limited. For molybdenum and BW for GA, a very weak inverted U-shape association was found.[102] The committee observed, however, that this study exclusively included subjects with complete covariate information, and the primary focus of the study revolved around examining mixtures of metals. Furthermore, the study was conducted within an impoverished urban population, likely above the average risk of exposure and intra-uterine growth retardation.

In a study on a sub-cohort selected from a larger birth cohort study in the USA, Kim et al. (2020) investigated whether exposure to metals, including Mo, negatively impacts intra-uterine growth.[103] The study included 130 women who experienced a preterm delivery and randomly selected controls (n=352 women), who were originally selected for a nested case-control study. Molybdenum and 16 other metals were measured in urinary samples collected at a median gestational age of 26 weeks. Median (IQR) molybdenum concentration, specific-gravity-corrected and weighted for case-control design, was 51.3 (37.1-69.7) ppb. Metal concentrations were modelled with various parameters of foetal growth assessed by ultrasound at 26 weeks (median) and 35 weeks (median) of pregnancy using linear mixed effects models, adjusted for multiple confounders. Linear regression analyses were used for associations with birthweight, birth length, and placental weight. In single metal analysis, no associations were

observed between molybdenum concentration and any of the outcomes. In models with additional adjustment for other metals, urinary interquartile molybdenum concentrations were associated with a 0.30 SD increase in femur length z-score (95% CI 0.08, 0.52), but no significant association was observed for the head circumference z-score.[103] The committee noted that the availability of the ultrasound measurement was selective, as the ultrasounds were taken at the participant's request or when abnormalities were suspected.

In the MADRES cohort, Howe et al. (2021) evaluated the associations between the exposure and foetal size at mid-pregnancy in a subset of 195 participants who enrolled prior to a routine ultrasound scan.[104] The methods of measurement and analyses were the same as the previous study of Howe et al., with a median (IQR) urinary molybdenum concentration of 57.4 (44.3-81.1) µg/L. Out the six and ten metals evaluated simultaneously in the primary and exploratory analysis, respectively, molybdenum ranked the highest as predictor of estimated foetal weight (EFW) and other foetal growth parameters, including abdominal circumference, biparietal diameter, femur length, and head circumference. A positive linear association was observed between molybdenum and EFW. When adjusting for the median levels of other metals, an increase in molybdenum from the 25th to 75th percentile was associated with a 0.114 (95% CI: 0.019, 0.247) SD higher EFW, equivalent to a 7.4 g increase in EFW. Furthermore, there was a 0.30 (95% CI: 0.05 - 0.56) SD difference in head circumference.[104] However, the biological significance of a 7.4 g increase in EFW during mid-pregnancy remains a subject of questioning, according to the committee. Furthermore, only subjects with complete covariate information were included, and the primary focus of this study was on mixtures of metals. An impoverished urban study population was used, likely with an above average at risk of exposure and intra-uterine growth retardation.

Karakis et al. (2021) performed an exploratory analysis using a cohort of 111 pregnant women of Arab-Bedouin origin and their offspring in Negev desert, Israel.[105] Associations between molybdenum (among other metals) in urine sampled just prior to delivery and adverse perinatal outcomes and other health problems of the offspring up to six years after birth were studied. The geometric mean of urinary molybdenum concentration was 7.23 ppb (95% CI 3.86 – 13.55 ppb). Molybdenum concentration (in quintiles) was found to be associated with behavioural/developmental disorders as reported in medical records (RR=1.86 per increment, p=0.016; the number of cases with a behavioural/developmental disorder was 7). Molybdenum concentration was not

associated with preterm delivery (RR=1.32, p=0.129), congenital malformations (RR=0.89, p=0.655), or other disorders.[105] Although an association with behavioural/-developmental disorders has been identified in this study, it remains unclear how the categories were defined and whether they might be subject to overreporting. Furthermore, the committee noted that the scientific value of this study is limited due to the relatively small study size, small number of cases, and the potential for residual confounding by, for example, other metals.

In an Australian prospective cohort study among pregnant women, McKeating et al. (2021) applied elemental metabolomics in plasma and urine to identify associations between elemental concentrations and pregnancy risk factors.[106] Plasma and urine samples were obtained from a cohort of 18-week pregnant women (N=128, age >18 years) from a hospital in Adelaide, Australia. ICP-MS was used to measure 27 plasma elements and 37 urinary elements, including molybdenum. The pregnancy outcomes were divided into 3 groups: 13 pre-term births, 10 small for gestational age (SGA) and 87 healthy infants (controls). The plasma molybdenum concentrations were 0.71 +/- 0.15 µg/L, 0.72 +/- 0.13 µg/L, and 0.89 +/- 0.32 µg/L, respectively. The urine concentrations were 4.12 +/- 2.1 ng/L, 3.34 +/- 0.98 ng/L, 4.66 +/- 2.34 ng/L, respectively. No differences were found in plasma and urine concentrations of molybdenum between preterm birth or SGA and control infants.[106] The committee noted that the analysis was not adjusted for potential confounding factors, plasma samples were not available for all participants, and the study power was limited due to a small sample size.

Tung et al. (2022) studied the associations between single and multiple placental metal concentrations (including molybdenum) and atypical neurobehavior in newborns in a prospective cohort study including 192 mother-infant pairs.[107] Due to the main aim of the cohort to understand aberrant foetal growth, the study included an overrepresentation of term infants who were either born large or small for gestational age. Placental samples were collected within 2 hours after delivery. The mean (interquartile range) placental molybdenum concentration was 6.76 ng/g (5.85-7.42 ng/g). Neurobehavioral performance was assessed with the NICU Network Neurobehavioral Scale (NNNS), administered by certified psychometrists 24 – 72 hours after birth, and divided into 5 profiles. Profile 5 indicated the most atypical neurobehavioral performance. Multivariate logistic regression analysis with adjustment for infant sex, maternal age, maternal race, pre-pregnancy BMI, and education status during pregnancy resulted in an OR of approximately 2 (with a wide 95% CI including

unity) for NNNS profile 5 with each doubling of placental molybdenum concentration. In a g-computation mixture analysis including 8 metals, cadmium was identified as the driving factor for the association, with molybdenum having a negligible negative weight.[107] Therefore, the committee did not judge the association for NNNS profile 5 as relevant.

Case-control studies

Yan et al. (2017) conducted a case-control study to investigate if 'essential trace metals', including molybdenum, protect against the risk of a neural tube defect.[108] This study included 191 women who had pregnancies complicated by neural tube defects in Shanxi Province and Hebei Province, China in 2003-2007. These cases were matched with 261 women from the same birthing hospital who delivered healthy infants and resided in the same country or city, with similar timing since their last menstrual period. Molybdenum and eight other metals were measured in hair segments grown just before and during early pregnancy. The median (IQR) molybdenum concentrations in hair were 0.071 ng/mg (0.062-0.084 ng/mg) for total NTD, 0.071 ng/mg (0.062-0.084 ng/mg) for anencephaly, 0.071 ng/mg (0.063-0.082 ng/mg) for spina bifida, 0.074 ng/mg (0.062-0.091 ng/mg) for encephalocele, and 0.075 ng/mg (0.063-0.088 ng/mg) in the control group. Associations were found for molybdenum concentrations, adjusted for multiple confounders and dichotomized by their corresponding medians in controls, with anencephaly (OR 0.51, 95%-CI 0.28-0.94), spina bifida (OR 0.54, 95%-CI 0.31-0.94), and total NTD (OR 0.64, 95%-CI 0.42-0.98). Dose-response analysis by quartiles of molybdenum concentrations showed statistically significantly lower NTD risk in the fourth quartile compared to the first quartile.[108] It should be noted that no multivariable analyses were conducted to assess co-exposure to other metals. Additionally, to maximize the sample size, matched pairs were separated for the analysis with unconditional logistic regression.

Associations between concentrations of 16 trace metals, including molybdenum, and infants small for gestational age (SGA) were investigated by Deyssenroth et al. (2018).[109] The metals were measured in maternal toe nail clippings collected on average 2.8 months post-partum from participants in the Rhode Island Child Health Study (n=195). Mean toenail molybdenum concentrations (SD) were 0.018 µg/g (0.032 µg/g). Logistic regression and weighted quantile sum regression were performed for molybdenum and panels of the 16 trace metals, respectively, adjusted for multiple confounders and corroborated by Bayesian kernel machine regression (BKMR). No

association was observed between molybdenum and SGA in the single metal model, and molybdenum did not contribute to the association between SGA and a metal mixture.[109]
The committee noted that exposure likely occurred between half a year and 1.5 years ago. Despite a slight protective effect being observed at high concentrations, it remains uncertain whether this occurred at a relevant time during pregnancy.

The associations between prenatal exposure to 22 metals, including molybdenum, and low birth weight (LBW) were investigated by Hou et al. (2019) in a nested case-control study in China.[110] This study included 246 women with LBW children and 406 women with normal birth weight children from a prospective birth cohort study. Maternal serum samples collected during prenatal examination were analysed by ICP-MS. In approximately 2/3 of the study population, both in the cases and control groups, serum collection took place during the second trimester of pregnancy (70.3% and 67.7%, respectively). However, some samples were taken in the first and third trimesters. The median molybdenum serum concentrations were 1.18 µg/L in the cases and 1.07 µg/L in the control group. In the single metal analysis, adjusted for multiple confounders, lower quartiles of serum molybdenum concentration were associated with a decreased risk of LBW compared to the highest quartile (P for trend = 0.018, OR 0.48 (95%-CI 0.30-0.77) for second quartile). In the multi-metal analysis, 15 metals, including molybdenum, were associated with a higher risk for LBW. However, no dose-response relationship could be established in cubic spline regression model. Furthermore, it should be noted that the gestational age at delivery was lower in cases than controls, as expected with LBW.[110]

Cross-sectional studies

Pi et al. (2019) examined the associations between concentrations of 6 metals including molybdenum in placental tissues and orofacial clefts (OFCs) in offspring in a rural population in northern China with a high prevalence of OFCs.[111] The study included 103 newborns and terminated foetuses with OFCs (cases) and 206 newborns without congenital malformations (controls). These groups were matched based on the mother's residence, date of last menstrual period, and sex of the newborn. Placental tissue was sampled after delivery or pregnancy termination, mostly during the 2nd or 3rd trimester. Median (IQR) placental molybdenum concentrations were higher in cases than controls, measuring 35.9 (31.7–41.8) and 32.1 (27.3–37.0) ng/g dry weight, respectively (p<0.001). The unadjusted association between molybdenum

concentrations above (≥33.6 ng/g) and below (<33.6 ng/g) median concentrations and the risk of OFCs with an odds ratio of 2.20 (1.36-3.58) nearly disappeared (OR 1.42, 95%-CI 0.78-2.59) after adjusting for multiple confounders, including gestational age.[111] It should be noted that the adjustment for gestational age may have resulted in an overadjustment. Furthermore, the analyses have not been adjusted for co-exposure to other metals.

In a cross-sectional study from Italy, metal concentrations in maternal serum collected during the 2nd trimester of pregnancy were compared between cases and controls by Troisi et al. (2019).[112] Cases were 111 pregnant women carrying a foetus diagnosed with a malformation (n=67) or chromosomal abnormality (n=44). Controls were 90 women with normally developed foetuses, recruited during second trimester routine anomaly scan. No differences in serum molybdenum concentrations were found between cases and controls (natural logarithm of the mean concentration +/- sd -3.09 +/- 1.10 µg/L), neither in a CNS group (n=17) including all CNS malformations with unknown aetiology nor in the 'other malformations' group (n=94) with unspecified malformations or chromosomal abnormalities.[112] The analyses were not adjusted for potential confounders.

The cross-sectional study by Ovayolu et al. (2020) in Turkey was performed to determine the levels of 14 trace elements and heavy metals, including molybdenum, in amniotic fluid of pregnant women collected around 20 weeks of gestation, and the associations with neural tube defects (NTDs).[113] The study included 36 pregnant women whose foetuses were complicated with NTDs (case group) and 39 pregnant women with unaffected foetuses (control group), matched for body mass index and gestational weeks. The amniotic fluid levels of molybdenum were measured using ICP-MS and compared between the two groups. Lower mean concentrations of molybdenum were observed in the NTD group than in the control group, measuring 1.11 µg/L (± 1.06) and 2.47 µg/L (± 1.92), respectively (p<0.001).[113] These results were not adjusted for potential confounders or co-exposure to other metals. This is particularly relevant since the cases were younger than controls and less frequently had a history of abortion.

Yin et al. (2020) studied the associations between placental metal concentrations and neural tube defects (NTDs) in a rural source population in China.[114] This study included 408 NTD cases with placental tissue available and 593 foetuses or newborns without structural malformations as controls from the same hospital and matched on mothers'

province of residence and date of last menstrual period, including controls selected for OFC cases. Placental median (IQR) molybdenum concentrations were higher in cases than controls, measuring 41.3 (32.8–51.2) and 32.8 (26.8–39.7) ng/g-dry weight, respectively (p<0.001). Molybdenum concentrations above the median of 35.7 ng/g were associated with a higher risk of NTDs versus lower concentrations, with an adjusted odds ratio of 3.73 (95%-CI 2.74–5.07), and similar results were observed for anencephaly and spina bifida separately. Dose-response relationships were seen with increasing ORs for quartiles of molybdenum concentration. Importantly, molybdenum did not have any effect in the BKMR analysis when all other metals were also included.[114]

Yin et al. (2020) studied associations between concentrations of six trace elements, including Mo, in serum samples collected during pregnancy or after birth and orofacial clefts (OFCs) in China.[115] The study included 130 women with foetuses or newborns with OFCs (cases) and 260 women with non-malformed foetuses or infants (controls), matched on province or city and first day of last menstrual period (±4 weeks). Median (IQR) molybdenum concentrations were lower in cases than controls, measuring 2.38 (1.76-2.94) and 2.82 (2.39–3.50) ng/ml, respectively (p<0.01). The adjusted odds ratio's for OFCs as a group, as well as for cleft lip with cleft palate (CLP) and cleft lip only (CLO) separately, decreased with increasing tertiles of molybdenum concentration (ORs ranging from 0.42 (2nd tertile compared to 1st tertile, CLP group) to 0.27 (3rd tertile compared to 1st tertile, CLO group), all 95% CI excluding unity; p for trend <0.01). Molybdenum concentrations were also inversely associated with the risk of OFCs in multi-metal analyses including adjustment for multiple confounders. It should be noted that the number of gestational weeks at blood sample collection differed substantially between cases (31.5% <28 weeks) and controls (78.1% ≥37 weeks).[115]

Gomez Roig et al. (2021) studied associations between placental concentration of molybdenum (among other elements), foetal growth and markers of placental function among 167 mother-infant pairs from Barcelona, Spain.[116] Prenatal Doppler ultrasound examinations were done at the beginning of the third trimester of pregnancy. On the basis of estimated foetal weight, the group was divided into 71 small foetuses (<10th percentile) and 96 normally grown foetuses (>10th percentile). Placental function was assessed by measuring the Pulsatility Index of the uterine artery, the umbilical artery and the middle cerebral artery. After delivery, placental molybdenum concentrations of 0.01+/-0.03 mg/kg (mean +/- sd) were determined using ICP-OES. No statistically significant (p>0.05) differences in placental molybdenum concentration were observed

between small and normally grown foetuses or placental function groups (>95th percentile vs. <5th percentile of the artery pulsatility index).[116] It should be noted that exposure was determined later (after delivery) than health outcome (at third trimester) or at the same time (pulsatility index arteries). Analyses for molybdenum were not adjusted for potential confounders. Dichotomisation of foetal weight (and artery PI) is probably not optimal for analyses in terms of statistical power. Furthermore, 71 out of 167 foetuses were SGA, while foetal weight was not part of the selection criteria.

In a cross-sectional study in the Shanxi province of northern China, Tian et al. (2021) studied associations between serum metal concentrations, including molybdenum and neural tube defects (NTD).[117] This study included 273 women with NTD-affected pregnancies (97 anencephaly, 127 spina bifida, 29 encephalocele, 20 NTD with other malformations). These cases were matched with 477 women with non-malformed foetuses or newborns from the same birthing hospital (controls), with similar timing since their last menstrual period (±4 weeks). Fasting and non-fasting blood samples were collected during pregnancy, at termination of pregnancy, or at delivery (cases 15% ≥37 weeks; controls 91% ≥37 weeks). [118] Median (IQR) molybdenum concentrations were lower in cases than controls, measuring 2.51 (1.43-3.07) and 2.66 (2.03–3.27) ng/ml, respectively (p=0.001). Statistical analysis using multilevel mixed-effects logistic regression, adjusted for multiple confounders, revealed that higher serum molybdenum concentrations were associated with a lower risk of any NTD for the 2nd (OR 0.48, 95% CI 0.26-0.90) and 3rd tertiles (0.54, 95%-CI 0.29-1.00) of molybdenum concentration compared to the 1st tertile. Similar results, but with wider confidence intervals, were seen for anencephaly and spina bifida separately. In multi-metal analysis, molybdenum was still inversely associated with risk of NTDs (adjusted OR 0.87, 95%-CI 0.80-0.94) for IQR increase in molybdenum concentration with other metals set at 50th percentile.[117] However, it is important to note that the number of gestational weeks (at which most of the blood samples were collected) differed substantially between cases (62.3% <28 weeks) and controls (90.7% ≥37 weeks). Furthermore, the committee noted that the determination of exposure occurred after the effects had already taken place.

In a cross-sectional study, Zhao et al. (2021) investigated associations between spot urinary molybdenum concentrations and ultrasound parameters of foetal growth at 22-28 weeks of gestation and offspring birth weight in 220 women in Hangzhou, China.[119] Multivariable linear regression analyses were applied with adjustment for multiple potential confounders. Creatinine-adjusted molybdenum concentration (geometric

mean, IQR: 66.1 µg/g, 47.0 - 87.8 µg/g) was associated with a 0.34 cm (95% CI 0.04 to 0.63) reduction in foetal abdominal circumference per unit increment and potentially with an 18.2 g (95% CI −4.2 to 40.5) lower estimated foetal weight. No associations were observed between molybdenum concentration and biparietal diameter, femur length, head circumference, and birth weight.[119] It should be noted that no other metals were taken into account.

7.2 Animal data

An overview of the *in vivo* studies on adverse effects on development is provided in Table A8 of annex A.

Jeter et al. (1954) administered doses of <1, 20, 80, or 140 ppm molybdenum (approximately <0.04, 0.9, 3.5, 6.2 mg molybdenum/kg bw/day, assuming a mean body weight of 425 g and a food intake of 18.75 g per day.) as disodium molybdate dihydrate in diets containing 5 ppm copper (normal copper content 1.8 ppm) to Long-Evans rats (N=4 or 8/sex/group) for about 20 weeks.[79] Age and body weights of rats at the start of the study were not given; only average weight gains at week 11. Depigmentation of the hair and alopecia were observed in some rats fed 20, 80 or 140 ppm dose groups. The average weight gain of male rats was statistically significantly decreased in the 20, 80 or 140 ppm dose groups, as well as in the females dosed 80 and 140 ppm. The average weight gains over the first eleven weeks were (controls: 176 g (males) 128 g (females), 80 ppm: 147 g (males) 105 g (females), 140 ppm: 80 g (males) 85 g (females). The average birth weight was 5.21 g in controls, 4.77 g (20 ppm), 4.72 g (80 ppm), 5.07 g (140 ppm). Number of dead pups at birth was 1 at 80 ppm and 11 at 140 ppm. Number of dying pups before 21 days was 7 in controls, 13 in 20 ppm group, 6 in 80 ppm group and 9 in 140 ppm group. The average pup weight at 21 days was 32.7 g in controls, 29.3 g in 20 ppm group, 28.3 g in 80 ppm group and 23.8 g in 140 ppm group.[79]

Schroeder et al. (1971) exposed five pairs of Charles River CD mice to 10 mg/L molybdenum (as molybdate; cation unknown) in deionized drinking water for up to six months, while the diet contained 0.45 ppm molybdenum (Assuming a mean water intake of 167 to 200 mL/kg bw/day and a food intake of 120 to 150 g/kg bw/day, the total intake of molybdenum per day approximates 1.7 to 2 mg/kg bw.).[80] Animals were allowed to breed freely during this period. Animals were at random selected from the first three litters to form the F1, and allowed to breed to form the F2 (period not

indicated). Animals of the first two F2 litters were selected to form the F3-generation. No mortality was observed in the F0-generation. Molybdenum did not affect the growth rate in the F0-generation. No other data on this generation are available. In the F1-generation, no differences between treatment group and controls were reported for number of litters, litter size and number of runts. Fifteen of the 238 F1 mice died early at 0.45 ppm molybdenum (not further specified). In the selected animals of the F1-generation, one female died in the treated group. The interval between the litters was increased (43 versus 28 days in controls), but the age at first litter was not affected. The number of F2 litters, litter size, and dead young were similar to controls. Five of the 26 litters were found dead compared to 0 out of 23 in controls. In the selected F2, four maternal deaths in the treated group were reported, and the age at first litter was increased from 62 to 79 days. No effect on interval between litters was found. The number of litters and litter size were decreased in treated animals. Four maternal deaths were found in the F3 generation. Also, four litters in the F3 were found dead in the treated group. The numbers of runts (11 versus 0 in controls) and dead young (34 versus 1 in controls) were increased.[80] There are no further details on the selection of the litters. The numbers of pairs selected in the F1 and F2 were not reported. No details on the dead pups and their incidence along different litters was reported for any of the generations. The study is poorly reported and no definite conclusion can be drawn based on the data available.

Fungwe et al. studied female Sprague-Dawley rats (N=21/group) that were given drinking water with 0, 5, 10, 50 and 100 mg/L (estimated at 0, 0.76, 1.5, 7.6, and 15 mg molybdenum/kg bw/day) molybdenum as sodium molybdate dihydrate from weanling onwards for 6 weeks.[81] Thereafter rats were exposed during three oestrus cycles before being mated with untreated males (N=15/ group) or sacrificed (N=6/group). The mated females remained exposed during gestation until necropsy on day 21. During the first 6 weeks of the study, no effects on body weight became apparent. During gestation, weight gain of the dams was statistically significantly decreased at 10, 50 and 100 mg/L, but these changes were attributed to reduced foetal weights. The number of resorptions was increased in females treated at 10 mg/L and above. Litter size did not differ between treatment groups and controls, but foetal weight and length were decreased at 10, 50 and 100 mg/L. Growth retardation was observed (less mature hepatic structure, delayed transfer of foetal haemopoiesis to bone marrow, delayed foetal oesophageal development, and myelination in the spinal cord) in the foetuses at 10 mg/L and above. Blood and hepatic enzymes of the dams were affected at 5 mg/L and above. Plasma ceruloplasmin was statistically significantly increased in

all gestating dams, but not in dams sacrificed after three oestrus cycles. Hepatic xanthine oxidase/dehydrogenase, and sulphite oxidase, were statistically significantly increased in all treated females in the study.[81]

Howell et al. (1993) studied the effect on the trace element status, and reproductive capacity of guinea pigs of ammonium molybdate (AM) and thiomolybdate (TM, presumably ammonium tetrathiomolybdate) in drinking water.[82] Mature female (N=8/dose) and male (untreated; 12 in total) Hartley albino guinea pigs, weighing around 500-600 g, were fed ad libitum a diet containing 212 µmol copper/kg diet. When each female entered the third oestrus cycle, males were introduced twice a day. Females of dose groups A (control), B (261 µmol AM/L), C (261 µmol TM/L), and D (130 µmol TM/L) received molybdenum compounds from the first day of the oestrus cycle onwards, whereas treatment of group E (261 µmol TM/L) and F (130 µmol TM/L) females was started immediately after mating. (Assuming a mean water intake of 100 to 170 mL/kg bw/day for guinea pigs, the units in µmol/L correspond to a daily intake of approximately 8.70 mg AM/kg bw (261 µmol/L), 11.55 mg TM/kg bw (261 µmol/L), and 5.75 mg/kg bw (130 µmol/L).) Subcutaneous oedema was found only in 1/8 and 4/8 female adult guinea pigs of the high TM dose groups, C and E. Upon X-ray examination, an ossified ridge in the mid shaft region of the femur was observed in the TM-dose groups (frequencies: 3/5, 0/7, 4/5, and 1/7 for groups C, D, E, and F, respectively), but not in the AM-treated animals nor in any of the pups. The reason for reporting the results for less than eight animals was not given, but it might be that animals that died (pregnant or non-pregnant) were excluded from examination. At birth, two animals of each litter were retained with the mother for a further six weeks. All dams and pups were X-rayed after they had been killed. Clinical signs observed in several dams of the high TM-dose groups included hair loss, transient diarrhoea, subcutaneous oedema, and mortality before or during pregnancy. No changes in ossified femur were observed in any of the pups. There appeared to be a reduced pregnancy rate in AM-treated females (4 out of 8 animals were pregnant), and an increased ‘aborted resorbing’ in high TM-dose females (group C). The mean number of pups born alive was reduced in groups B, C, D and E, but not in group F. Pup body weight was slightly decreased at birth in the TM-treated groups. Six weeks after birth, body weight gain of group C pups (high TM dose, 317,4 g (SE 26,1)) was lower as compared to controls (364,2 g (SE 31,3)). Administration of AM or TM usually resulted in an increase in the concentration of molybdenum in the organs examined (the liver, kidneys, femur, and brain). This increase was statistically significant in the liver, kidneys, and femur at all ages in the group given AM; and, in the liver and kidneys at

birth in all groups given TM with the exception of the liver in group E. However, the concentration of molybdenum was statistically significantly depressed in the femur of the pups from group F killed at six weeks.[82]

In a dose-range finding study, sodium molybdate dihydrate was administered in the diet ad libitum to pregnant Sprague-Dawley rats (N=10/ group) at doses of 0, 1, 5, 10 and 20 mg molybdenum/kg bw/day from Days 6 to 20 of gestation.[120] At gestation day 20, the animals were sacrificed and gross necropsy was performed. No molybdenum-related developmental toxicity (pre and post implantation loss, foetal numbers, sex ratio, body weights and or foetal external malformations) was observed.[120]

Based on the previous outcome of IMOA 2012,[120] the study was repeated with higher doses (IMOA 2013).[121] Sodium molybdate dihydrate was given to maternal Sprague Dawley rats (N=25/ group) via the diet at doses of 0, 3, 10, 20 and 40 mg molybdenum/kg bw/day from Days 6 to 20 of gestation. At gestation day 20, the animals were sacrificed and gross necropsy was performed. No treatment-related effects were observed on maternal body weight, weight changes, feed consumption, clinical observations, pregnancy indices or maternal organ weights. Also no treatment related effects were observed regarding numbers of ovarian corpora lutea, uterine implantation sites and losses, number of foetuses, foetal sex ratios, foetal body weights, foetal external, visceral or skeletal malformations or variations in the foetuses per females.[121] The committee cannot make a final conclusion on the present and the previous study, since a lack of maternal toxicity in combination with a lack of developmental effects may indicate that the chosen exposure levels were too low to draw conclusions on classification or labelling. In that case, and according to OECD TG 414 (prenatal developmental toxicity study), further investigations are needed.

Sodium molybdate dihydrate in the diet was tested for developmental toxicity in Sprague Dawley rats in accordance with OECD TG 414.[90] Dose levels of 0, 3, 10, 20 and 40 mg molybdenum /kg bw/day were administered from gestation day (GD) 6 to GD 20. The dose levels were chosen based on a range-finder study in rats given sodium molybdate dihydrate. In this range-finding study, no treatment-related maternal effects or developmental toxicity were observed up to 20 mg Mo/kg bw/day (the highest dose tested). In the main study, no adverse effects were observed at any dose level on the dams, or on embryofoetal survival, foetal bodyweight, or development, with no increase in malformations or variations. Significant increases in serum and tissue molybdenum and copper levels were observed but no related toxicity was observed.[90]

Given the absence of effects at any dose level, the committee considered the applied doses as too low.

In a two-generation study, performed according to OECD TG 416, groups of 24 male and 24 female Sprague-Dawley rats were administered sodium molybdate dihydrate at 0, 5, 17, or 40 mg/kg bw/day in the drinking water or 40 mg/kg bw/day in the diet over two generations to assess reproductive toxicity (see section 6.2).[84] Serum levels of molybdenum and copper were increased in a dose-related manner for which molybdenum serum concentrations were slightly higher when given 40 mg/kg bw/ day in the diet as compared to the same dose via drinking water. Limited systemic toxicity, including decreased body weight, food consumption (males only) and water consumption, was observed among both sexes given 40 mg/kg bw/day in the diet. No adverse effect on development was observed at any dose level in either generation after exposure in drinking water.[84] Therefore, the committee considered the applied doses too low for evaluation for developmental toxicity.

A developmental mouse study estimated the possible induction of genotoxicity and foetal abnormalities, especially foetal malformations and skeletal abnormalities, by molybdenum nanoparticle administration.[122] Molybdenum nanoparticles with a size less than 100 nm were suspended in deionized distilled water and were orally administered to 5 pregnant mice per group at dose levels of 500 (group 1 and 2) or 750 mg/kg bw (group 3 and 4) from the 1st up to the 17th day of pregnancy (groups 1 and 3) or from the 9th up to the 17th day of pregnancy (groups 2 and 4). Oral administration of molybdenum nanoparticles resulted in significant decreases in the maternal body weight gain, the number and length of foetuses (no quantitative data) as well as skeletal abnormalities, mainly less ossification and less chondrification. Administration of molybdenum nanoparticles also caused DNA damage induction (as measured by a Comet assay) and elevated expression of levels of p53, a gene involved in maintaining the genomic stability and cell differentiation in both maternal and foetal tissues. The expression levels of E-Cad and N-Cad genes that control skeleton development were increased in the tissues of female mice administered molybdenum nanoparticles and their foetuses.[122] The committee noted a low number of animals was tested per group and it was unclear how many foetuses were assessed for adversities. The figures containing the relevant data were difficult to read and important information on the statistics was missing. The choice in test groups limited the possibility to deduce a dose-response relationship, because of differences in exposure duration and doses (Group 1: exposure from day 1-17; 500 mg/kg bw/day. Group 2: exposure from day 9-

17; 500 mg/kg bw/day. Group 3: exposure from day 1-17; 750 mg/kg bw/day. Group 4: exposure from day 9-17; 750 mg/kg bw/day.). Lastly, the observed effects could be attributable to the release of molybdate ions or to the nanoparticles, which due to their size may interfere with cellular structures.

A prenatal developmental toxicity study (OECD TG 414) with sodium molybdate was performed in rats. Preliminary to this study, a tolerance study and a dose range finder study were performed. The tolerance study of sodium molybdate via oral gavage demonstrated marked toxicity at 300 mg/kg bw/day and mortality at higher doses.[83] In the dose range finder study, pregnant rats were exposed to 300 and 400 mg/kg bw/day (equivalent to 120 and 160 mg molybdenum/kg bw/day, respectively) sodium molybdate via diet, from Days 6-20 of gestation.[83] Excessive toxicity was observed at 400 mg/kg bw/day and therefore 300 mg/kg bw/day was selected as the high dose. The substance was less tolerated via gavage as compared to diet and the OECD TG 414 study was therefore performed via diet.

In the adjusted OECD TG 414 study performed by Aveyard et al., Sprague Dawley rats were given 0, 200 or 300 mg/kg bw/day (0, 80 or 120 mg /kg bw/day expressed as molybdenum, determined through a dose-range finding study) of sodium molybdate dihydrate via diet at Days 6-20 of gestation to investigate developmental toxicity.[123, 124] On gestation day 21, part of the females rats were euthanized (caesarean section animals). The other part was assigned to two littering groups (controls and high dose) to allow delivery and weaning of pups (littering animals). Dose-dependent moderate to marked maternal toxicity was observed at both dose levels, including adverse clinical observations, reductions in maternal weight gain and food intake over the administration period, and reduced corrected (for uterine content) body weight at gestation Day 21. Liver weights were reduced and test item-related microscopic changes were present in the kidney and liver. Total placental weight per litter was reduced at 300 mg/kg bw/day, compared to controls. The relative weights were not significantly different.
In animals that underwent a caesarean section, dose-dependent reduction in foetal weight was observed. In littering animals, mean pup weights (combined sexes) were significantly lower in the highest dose group than in the control group. No effects were seen on viability indices, lactation indices, litter sex ratio and litter size. The mean anogenital distance was reduced by 6% in males of the 300 mg/kg bw/day dosed animals compared to the controls, and after adjustment for foetal body weight.
The incidence of external, visceral and skeletal foetal malformations and variations was

not affected by sodium molybdate dihydrate. The slight differences in the ossification status of foetuses in the 300 mg/kg bw/day group were confirmed as transient by skeletal examination of pups at Day 21 post-partum, and are consistent with the reduced foetal weight, associated with the marked maternal toxicity observed at this dose level.[123, 124] The committee considered the effects seen on anogenital distance as relatively small. A lower anogenital distance could be secondary to a reduction in foetal size, so the observed reduction in foetal body weight may have made the examination of the anogenital distance more challenging, as well as the interpretation of the results.

In a study by Murray et al (2023) female weanling Sprague-Dawley rats were administered sodium molybdate dihydrate in drinking water.[85] This study aimed to repeat and confirm the findings that were previously described by Fungwe et al. (1990) for both developmental and reproductive toxicity.[81] The chosen dose levels of 0, 20 or 40 mg molybdenum/ kg bw/day differed from those chosen by Fungwe et al and were based on the NOAEL that the authors deduced in 2014 in a developmental toxicity study.[90] Because of the hypothesis that the difference in copper diets caused the differences in findings between Fungwe et al. and the guideline studies, the copper concentration was accommodated to a concentration of 6.2 ppm in the rats diet, which is similar to the concentration that Fungwe et al used. Although the authors aimed to replicate the study, the authors describe some differences in the experimental design between the two studies related to the dose levels, group sizes, and exposure duration. Murray et al did not find statistically significant effects on total, early or late resorptions. Also, Murray et al. did not find sodium molybdate dihydrate related effects on foetal body weight or foetal malformations or variations compared to controls at 20 or 40 mg molybdenum/kg bw/day.[85]

7.3 Evaluation of the data

Epidemiological studies

Two studies found an inverse association between molybdenum exposure and growth and measures of body weight in offspring.

One prospective cohort study observed that boys were found to have a reduced head circumference associated with paternal molybdenum concentration, but no associations were found between maternal or paternal molybdenum concentration in urine and gestational age, birth weight, birth length, ponderal index, and infant sex.[99, 100] However, it should be noted that the molybdenum concentration in the samples were low relative to the U.S. population values for 2005–2010, the study participants were assumed to be at risk of persistent organic pollutants exposure, and no correction for multiple testing was conducted in this analysis.

One cross-sectional study showed an inverse association between creatinine-adjusted molybdenum concentration in urine and foetal abdominal circumference, but no associations were observed between molybdenum concentration and biparietal diameter, femur length, head circumference, and birth weight.[119] However, co-exposure to other metals was not taken into account.

Eight epidemiological studies found inconclusive evidence concerning the association between molybdenum exposure and adverse effects on growth and body weight in offspring.

One prospective cohort study found that urinary molybdenum concentrations were associated with an increase in femur length z-score when adjusted for other metals.[103] It should be noted that the availability of the ultrasound measurement was selective. A prospective cohort study by Howe et al. (2020) found a weak inverted U-shape association between urinary concentrations of molybdenum and birth weight for gestational age.[102] Another study of Howe et al. found a positive linear association between urinary concentrations of molybdenum and estimated foetal weight and an increase in head circumference.[104] Single metal analysis in a nested case-control study showed that lower quartiles of serum molybdenum concentration were associated with a decreased risk of low birth weight compared to the highest, when adjusted for multiple confounders.[110]

A prospective cohort study observed no association between urinary molybdenum concentrations and birthweight, birth length, and head circumference, but it should be noted this study has several limitations, as described in the study summary.[97]

Another prospective cohort study and a case-control observed no association between urinary concentrations of molybdenum and SGA,[106, 109] but both of these studies had relatively small population sizes, and potential confounders cannot be ruled out.

One cross-sectional study observed no differences in placental molybdenum concentration between small and normally grown foetuses or placental function groups, but the analysis for molybdenum was not adjusted for potential confounders.[116]

Several epidemiological studies addressed other developmental parameters. Three prospective cohort studies reported associations between prenatal molybdenum exposure and neurodevelopment parameters.

One study found that a doubling of urinary molybdenum concentration in the third trimester was associated with a lower index on the psychomotor subscale of the Bailey's scale, but this effect was found on a sublevel of a subanalysis and the clinical relevance of this inverse association was poorly defined and therefore questionable.[98]

Another prospective cohort study also found that urinary concentrations of molybdenum were associated with behavioural/developmental disorders.[105] However, overreporting cannot be excluded be excluded and the statistical power is limited.

One study found that each doubling of placental molybdenum concentration resulted in an increased OR for NICU Network Neurobehavioral Scale profile 5, for which the co-exposure to other metals was taken into account. However, smoking was not accounted for as a potential confounder, less than 200 mother-infant pairs were evaluated, and the readout was measured two to three days after birth, which has been shown to have limited predictive ability for later neurodevelopment.[107]

One cross-sectional study found that increased concentrations of molybdenum in placental tissue were associated with a higher risk for neural tube defect (NTDs) in a multivariable logistic regression model, as well as dose-response relationships with increasing ORs for quartiles of molybdenum concentration.[114] However, molybdenum did not have any effect in the Bayesian kernel machine regression analysis when other

metals were included. Furthermore, placental molybdenum concentrations may not reflect the concentrations during the critical period of neural tube development, because tissue sampling occurred mostly in the second or third trimester. There is also a potential overadjustment by gestational age, which should not be included as a confounder.

On the other hand, one case-control and two cross-sectional studies found an inverse association between concentrations of molybdenum in maternal hair and NTDs. Dose-response analyses in a case-control study showed that increasing quartiles of molybdenum concentrations were associated with decreasing trends in adjusted ORs with for total NTD, anencephaly, and spina bifida, when adjusted for multiple confounders.[108] Ovayolu et al. (2020) observed lower mean concentrations of molybdenum in amniotic fluid in the NTD group than in the control group, but these results were not adjusted for potential confounders or co-exposure to other metals.[113] Tian et al. (2021) found that serum molybdenum concentrations were associated with a lower risk of NTDs for the 2nd and 3rd tertiles of molybdenum concentration, respectively, compared to the 1st tertile.[117] Similar results, but with wider confidence intervals, were seen for anencephaly and spina bifida separately. In confounding-adjusted multi-metal analysis, the molybdenum concentration was still inversely associated with risk of NTDs.[117]

Two cross-sectional studies investigated the association between placental molybdenum concentrations and orofacial clefts (OFC). Pi et al. (2019) found an unadjusted association between molybdenum and the risk of OFCs, but this effect nearly disappeared after adjusting for multiple confounders, including gestational age.[111] On the other hand, Yin et al. (2020) observed that molybdenum concentrations were inversely associated with the adjusted odds ratio's for OFCs as a group, as well as for cleft lip with cleft palate and cleft lip only separately.[115] Molybdenum concentrations were also inversely associated with the risk of OFCs in multi-metal analyses including adjustment for multiple confounders.[115]

Animal data

The studies published by IMOA did not show maternal toxicity nor developmental effects. The committee evaluated the chosen exposure levels as being too low to induce adverse health effects.[88, 89, 121] Three poorly reported animal studies indicated developmental effects, but at the presence of maternal toxicity.[79, 80, 82] Fungwe et al (1990) observed increased numbers of resorptions and decreased foetal weight and

lengths, also at the presence of maternal toxicity by means of decreased weight gain of the dams.[81] However, this decreased maternal weight could be attributable to the weight loss of the progeny. The study performed by Murray et al. in 2023 aimed to repeat the study of Fungwe et al. and could not confirm the developmental effects of decreased foetal weight and length and increased number of resorptions.[85]

One non-guideline study with molybdenum metal and three guideline studies with molybdate were available. The non-guideline study evaluated the effects on foetal external and skeletal morphology after oral administration of molybdenum nanoparticles (<100 nm) to pregnant mice.[122] The authors reported an increased incidence of skeletal abnormalities, but the study was poorly reported as the figures containing the relevant data were difficult to read and important information on the statistics was missing. Additionally, as molybdenum nanoparticles were used as the test item, this further complicates the understanding of its effects. Moreover, further details of the test item and species were not reported. The choice in test groups made it more difficult to deduce a dose-response relationship, because of differences in exposure duration and doses.[122]

Two of the guideline studies evaluated the effects of sodium molybdate dihydrate in a developmental toxicity study and a two-generation study.[84, 90] The dose levels of these studies seemed to be too low to be able to assess developmental toxicity. The third guideline study by Aveyard et al. evaluated the effects of sodium molybdate in a developmental toxicity study at higher dose levels of 80 and 120 mg /kg bw/day.[123] Although a reduction on foetal body weight was detected, this occurred at doses where maternal toxicity was observed. The effects seen on anogenital distance were relatively small. Additionally, a lower anogenital distance could be secondary to a reduction in foetal size, so the observed reduction in foetal body weight may have made the examination of the anogenital distance more challenging, as well as the interpretation of the results.

7.4 Conclusion

Human data

All epidemiological studies evaluated potential effects at relatively low exposure concentrations. No occupational studies were available for which exposures are typically measured at higher concentrations. The human evidence that was available

can be divided into the following groups: growth and body weight in offspring, neurodevelopment, and neural tube defects / orofacial clefts.

Considering molybdenum exposure and growth and body weight in offspring, two studies found small associations. One study found a small association with a decreased head circumference in a subanalysis within a subgroup, while another study found an association for molybdenum and abdominal circumference as the only parameter. These marginal findings in combination with eight studies that found inconclusive evidence concerning the association between molybdenum exposure and growth and body weight of the offspring are reason for the committee to conclude that molybdenum has an unconvincing association with growth and body weight and that insufficient available data exist to be conclusive about a relation.

As addressed in the evaluation, each of the three neurodevelopmental studies had several shortcomings. Furthermore, no association was found between molybdenum and neurodevelopmental effects after adjustment for multiple metals. Overall, studies evaluating molybdenum and neurodevelopmental effects were insufficient for classification due to low exposure levels.

The results of neural tube defects and orofacial clefts did not provide sufficient evidence, because the tested molybdenum concentrations were too low to draw firm conclusions. Pi et al. (2019) found no effect on orofacial clefts after adjustment for confounders and Yin et al. (2020) similarly found no effect on neural tube defects after adjustment for confounding. However, several other studies found inverse associations between molybdenum and neural tube defects and orofacial clefts.

Overall, the available human data provided no sufficient evidence for a classification. The molybdenum concentrations to evaluate effects on growth and body weight in offspring were in the lower ranges, and potentially did not capture exposure levels in occupational settings. Furthermore, multiple null studies existed. Sufficient evidence for effects on neurodevelopment were absent and no neural tube closure effects were observed within the measured concentrations.

Animal data

The study of Fungwe et al. found adverse effects such as decreased foetal weight and length, and increased number of resorptions.[81] However, the repeated study in 2023 did not confirm these developmental effects.[85]
The results of two poorly reported studies in mice and guinea pigs indicated developmental effects in the presence of maternal toxicity.[80, 82] One other study reported an increased incidence of skeletal abnormalities, but lacked thorough reporting, and used molybdenum nanoparticles as the test item, which complicates the understanding of the effects.[122]
Most available animal studies were considered to have used too low doses to cause adverse health effects (maximum 40 mg/kg bw/day). The study by Aveyard, however, did show marked maternal toxicity at doses of 80 and 120 mg/kg bw/day, but no other effects on development than a reduced foetal body weight were observed.[123] Based on the study by Aveyard, it can be concluded that no effects on development are expected at doses that do not show maternal toxicity.

Overall conclusion

Most epidemiological studies did not show increased risks and the studies that did indicate increased risks had methodological limitations. In addition, some uncertainty in the evidence from poorly reported animal studies still exists, and additional information is missing, such as developmental studies in a non-rodent species. Taken together, the committee concludes that the data available do not justify classification for effects on development.

8 Adverse effects on or via lactation

8.1 Adverse effects on lactation

No information was found for adverse effects of molybdenum and selected inorganic molybdenum compounds on lactation.

8.2 Adverse effects via lactation

Human data

Molybdenum is present in human breast milk, with mean concentrations ranging between 0.02 to 72 µg/L (see also chapter 4.2). The IARC monographs (volume 118) on molybdenum trioxide mentioned molybdenum contents in human breastmilk ranging from <0.1 µg/L to > 60 µg/L.[125] Molybdenum concentrations in human milk appear to be highest during the first few days of breastfeeding, and decrease during the course of lactation.[44] The Health Council mentioned two studies regarding effects on lactation in the previous molybdenum report in 2013.[4] Aquilio et al. (1996) detected molybdenum levels of 6.8 µg/L in human breast milk.[36] Another study by Al-Saleh et al. (2004) reported levels of 13±1 µg/L in maternal venous blood at delivery (N=17).[126]

Mandiá et al. (2021) studied molybdenum concentrations (among other elements) in human milk from nursing mothers from Santiago de Compostela, Spain and their associations with selected medical factors.[127] Human milk samples were obtained at three time points from 70 mothers of full-term newborns: colostrum during the first 3–4 days of lactation, intermediate milk up to 7–10 days of lactation and later mature milk up to 6 months after birth. In addition, samples of later mature milk were also obtained from 100 mothers of premature newborns. A questionnaire included medical factors of the mother during pregnancy and birth weight of the newborn. Molybdenum concentrations in milk from full-term mothers decreased as lactation progressed. Mean molybdenum concentrations in colostrum were 1.88 µg/L (95%CI 1.47-2.29 µg/L) and in intermediate milk 1.22 µg/L (95%CI 0.82-1.76 µg/L). Mean molybdenum concentrations in later mature milk were 0.96 (95%CI 0.68-1.25 µg/L) and 0.70 µg/L (95%CI 0.47-0.94 µg/L) from mothers of full-term and preterm newborns, respectively. In infant formula mean molybdenum levels varied between 20 and 35 µg/L depending on the type of formula with lower limits of 5 and upper of around 40 µg/L. Associations between absolute change means in trace element concentrations variables such as

birth weight and maternal weight gain were evaluated using linear regression models, with change represented as a coefficient.[127] The committee concluded that based on the obtained coefficients, associations were not found.

Wappelhorst et al. (2002) compared the element intake of nursing mothers and element content of human milk.[43] The mean intake of molybdenum was 132 ± 60 (sd) µg/day (2.07 µg/kg/day) and the mean milk concentration of the same group of women was 0.72 µg/L (minimum 0.27- maximum 1.62).[43]

Breast milk concentrations of 32 metals and elements were determined in early lactation (days 14-21) in a random sample of first time Swedish mothers (n = 60), collected in 2002-2009.[128] The elements were measured using ICP-MS. The mean molybdenum concentration in breast milk was 3.5 µg/L (sd ± 2.7); the median concentration was 2.4 µg/L (range: 0.8 – 12 µg/L). The results were compared to concentrations found in Swedish women in a WHO study from 1989. The median molybdenum concentration in milk in the WHO study was 0.40 µg/L (range: 0 – 5.9 µg/L). In this WHO study, samples were taken at about three months after the birth of the baby, instead of 2-3 weeks after birth, which could explain the difference in median concentrations.[128]

In a Japanese study, breast milk trace element concentrations were determined at 1 and 3 months postpartum using ICP-MS.[129] Samples were collected from 79 Japanese healthy mothers who gave birth to a single infant after 37 weeks of gestation. The mean molybdenum concentration in breast milk was 1.0 (sd ±0.9) and 1.1 (sd ± 1.1) µg/dL (10 and 11 µg/L) at 1 and 3 months postpartum respectively. The median concentration for 1-month postpartum human milk was 0.7 µg/dL (7 µg/L) with 25 and 75 percentiles of 0.3 µg/dL and 1.3 µg/dL, respectively. For 3-month postpartum milk this was 0.7 µg/dL (7 µg/L) with 25 and 75 percentiles of 0.3 µg/dL and 1.4 µg/dL, respectively. Maximum concentrations of molybdenum in human milk were not indicated.[129]

Trace elements, including molybdenum, were determined in human breast milk from Jordanian mothers (n=76) and measured by ICP-MS.[130] The mean molybdenum concentration was 32.6 µg/L (sd ± 28) and the median concentration was 25.0 µg/L, with an overall range of 2.70-236 µg/L.[130] The committee noted that the maximum concentration of 236 µg/L is at least 20 fold higher than other maximum values reported in other studies. Additionally, the median value is also higher compared to

those found in other studies. The cause of these high maximum molybdenum concentrations is unknown to the committee.

Intra- and inter-day variation of elements in breast milk in 11 women at 12 sampling points over three days by ICP-MS.[131] The median concentration of molybdenum was 1.5 ng/g, with a minimum of 0.18 and maximum of 12 ng/g.[131]

Human milk samples were collected pre- and post- every feed in a 24-hour period from 11 mothers and analysed for elements by ICP-MS.[132] Pre-feed concentrations of molybdenum in milk were 0.48 ± 0.28 (SEM) µg/L; post-feed concentrations were 1.20 ± 0.28 (SEM) µg/L. Although exact numbers were not indicated, maximum concentrations of molybdenum in pre- and postfeed milk were found to be 4 to 5 µg/L.[132]

Animal data

In an OECD TG 416 two-generation study, groups of 24 male and 24 female Sprague-Dawley rats were administered sodium molybdate dihydrate at 0, 5, 17, or 40 mg molybdenum /kg bw/day in the drinking water or 40 mg molybdenum /kg bw/day in the diet to assess reproductive toxicity.[84] This study is also described in section 6.2 (fertility) and 7.2 (development). No adverse effects on the pups during lactation were observed. Viability index, lactation index, % male pups per litter, live litter size and pup weight were not affected by sodium molybdate. Overall, no adverse effects on reproductive function or development were observed at any dose level in either generation. The committee considered the applied doses too low for evaluation for sexual function and fertility and developmental toxicity.

In the supplementary OECD 414 prenatal developmental toxicity study by Aveyard et al. Sprague Dawley rats were given 0, 200 or 300 mg/kg bw/day (0, 80 or 120 mg /kg bw/day) of sodium molybdate dihydrate via diet at Days 6-20 of gestation to investigate developmental toxicity.[123, 124] On gestation day 21, part of the females rats were euthanized (caesarean section animals). The other part was assigned to two littering groups (controls and high dose) to allow delivery and weaning of pups (littering animals). No effects were observed on nursing or nesting behaviours, pregnancy rate, implantation rate, post-implantation loss, litter size, % male pups per litter, pup viability, pup clinical conditions or growth. At each post-natal interval measured, the mean pup body weights in the 120 mg /kg bw/day exposed group were significantly lower compared to controls but pup growth rate exceeded controls as the pup weight were 19.2% lower than controls at birth but only 9.4% lower by post-natal day 21.

8.3 Evaluation of the data

The committee aimed to make a risk assessment for molybdenum via lactation. However, effects of molybdenum on lactating infants via breast milk are unknown, as information is lacking. Infant formula contained intentionally added molybdenum levels and were measured to be 20-35 µg/L by Mandiá et al.[127] This value was higher than the concentration that was measured in the majority of the studies described in section 8.2. In addition, the WHO guideline value of molybdenum in drinking water of 0.07 mg/liter (=70 µg/L),[133] which is higher than the majority of the measured molybdenum levels in breast milk. Lastly, no adequate reference value exists for comparing the observed molybdenum levels and is explained in the following paragraphs.

The Scientific Committee of Food (SCF), a committee of the EFSA, previously calculated an upper limit of 600 µg/day for adults (= 0.01 mg/kg bw /day), including pregnant and lactating women.[134] This UL was extrapolated to an UL of 100 µg/day for young children of 1 to 3 years old. An extrapolation to younger infants was not possible according to the SCF. The SCF indicated that there were no adequate human data available to establish an UL. Therefore, the study by Fungwe et al., 1990 was accounted for as the pivotal animal study, because of the effects on particularly foetal development.[134, 135] The committee noted that the study by Fungwe et al. could not be reproduced by Jay Murray et al. (2023), and SCF could not extrapolate an UL for infants aged 0-1 years old. This makes the proposed UL values by SCF not adequate as a reference value for the purpose of the committee.

The EFSA also referred to the by UK COMA derived safe intake ranges based on evidence from breastfed infants.[135] This range was based on a study by Casey and Neville, who collected human milk from 13 women and measured molybdenum levels (mean +/- sd) of 15.0 +/- 6.1 ng/mL on day 1 and 1-2 ng/mL (sd not given) after 1 month.[39] The derived safe intake ranges encompassed intakes between 0.5-1.5 µg/kg bw /day.[136] Assuming an average body weight of 4.5 kg and an average milk intake of 900 mL, the committee translated this range to 2.03-6.08 µg/L. The rationale for the derived safe intake ranges was not clear to the committee. This range is lower than the levels present in infant formula.

The lowest UL the US Institute of Medicine deduced among all age groups was the one for children aged 1-3 years of 300 µg/day. The NIH refers to the same value for 1-3 years old children. It was not possible to establish an UL for infants 0-1 years and they

stated that the source of intake should be from food and formula only.[137] The International Molybdenum Association also refers to this value.[138]

8.4 Conclusion

Overall, the committee found no relevant scientific data to draw a conclusion on effects on or via lactation as no effects of molybdenum in breastmilk on the development of offspring were assessed in any of the available studies.

9 Conclusions on classification and labelling

The committee recommends classification according to Regulation (EC) 1272/2008 of the European Union. For molybdenum and selected inorganic molybdenum compounds, the committee recommends the following classifications:

Proposed classification for fertility
Category 2 (Suspected human reproductive toxicant (H361f)).

Proposed classification for developmental toxicity
Classification for effects on developmental toxicity is not justified based on the available data.

Proposed labelling for effects on or via lactation
Classification for effects on or via lactation is not indicated because of a lack of relevant data.

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Committee

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A Supplementary tables

Fertility – human data

Table A1 Summary of epidemiological cross-sectional studies relevant for male sexual function and fertility.

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Meeker et al., 2008 [69] Cross-sectional study 219 men (18-55 years) recruited through two fertility clinics	Semen samples were collected using standard protocols	Semen quality (sperm count, sperm concentration, percent motile sperm, and sperm morphology) Metals in blood	Molybdenum-dependent decreases in sperm concentration and normal morphology OR for sperm concentration (95% CI): metal percentile 70-85th, 2.23 (95% CI, 0.66-7.60); metal percentile >85th, 6.26 (95% CI, 1.57-25.0). OR for sperm morphology: metal percentile 70-85th, 0.91 (95% CI, 0.37-2.24); metal percentile >85th, 3.44 (95% CI, 1.23-9.67).	Adjustment for age, current smoking, and the impact of multiple metals on semen quality simultaneously.	
Meeker et al., 2010 [70] Cross-sectional study 219 men recruited (18-55 years) through two fertility clinics	Blood samples were collected	Reproductive hormone levels (serum FSH, LH, inhibin B, testosterone, and SHBG)	Significant inverse trend between molybdenum concentrations in blood and testosterone levels,	Considering multiple metals and other potentially important covariates	

			also when correcting for exposure to other metals. Regression coefficient (95% CI): metal percentile 70th-85th, -18.5 (-53.3, 16.3) metal percentile 85th, -55.9 (-92.5, -19.3) High molybdenum was associated with a 37% reduction in spermatogenesis (relative to the population median level) among men with low zinc.		
Guzikowski et al., 2015 [139] Cross-sectional study 34 men (26-42 years) from primary infertile couples in the rural area of Opole, Poland January-June 2009 Inclusion criteria: - regular unprotected intercourse for at least 12 months without conception - no previous fertility treatment	Semen samples were collected after abstinence period of 5 days Concentration of Mo and 8 other metals determined using ICP-MS with time-of-flight analyser LOD/LOQ not reported	Semen quality according to WHO criteria: - volume - pH (not reported) - sperm count - sperm motility - sperm morphology Statistical analysis - Pearson's correlation coefficients between Mo	23 men with one or more deviating values for sperm quality parameters: - sperm concentration <20×10^6/ml - <50% motile sperm - <15% normal forms 11 men with sperm quality parameter values in the normal range	Confounding is possible since no other factors were considered in crude correlation analyses	Statistical analysis and reporting are poor; some descriptions in the results section do not match the tables

- no known causes of infertility in patient r partner		concentration and semen quality parameters	Concentrations of Mo were correlated with the concentrations of all other metals ($p<0.05$) except zinc and with sperm motility ($p=0.016$) Pearson's correlation coefficients for Mo concentration with semen parameters (N=34): - Sperm count: r=0.32 ($p>0.05$) - Sperm motility: r=0.19 ($p>0.05$) - Sperm morphology: r=0.15 ($p>0.05$)		
Lewis & Meeker, 2015 [140] Cross-sectional study United States (general population) 484 men, aged 18-55 years, participating in NHANES 2011-2012	Urine samples were collected Concentration of Mo (µg/L) and 5 other metals (+3 metals in serum) determined using ICP-MS LOD 0.99 µg/L	Blood samples were collected Serum testosterone was measured with HPLC-MS/MS Statistical analysis - Associations between urinary Mo concentration	Urinary Mo detected in all 484 urine samples (median 46.05, IQR 23.50-76,70 µg/L) Association between urinary Mo modelled *continuously* and testosterone: -4.26 (CI -7.70, -0.69)	Selection bias cannot be ruled out because a large part of the study population was excluded from the analysis (n=1367).	Distribution and other descriptive results on serum testosterone levels not provided.

		and log-transformed serum testosterone concentration assessed using multivariable linear regression models - Mo concentration used [1] continuously (µg/L); [2] in quartiles; and [3] in quintiles - All measures of association were expressed as % change in serum testosterone concentration associated with a doubling (100% increase) in Mo concentration and adjusted for age, BMI, income, race, serum cotinine, and urinary creatinine	Association between urinary Mo in *quartiles* and testosterone: ±10% lower serum testosterone in Q2, Q3, and Q4 compared with Q1 of urinary Mo level. P for inverse trend 0.107 Association between urinary Mo in *quintiles* and testosterone: p for inverse trend 0.020. More details on regression estimates not provided.		
Skalnaya et al., 2015 [141] Cross-sectional study Orenburg, Russia 148 men Study period not reported, but < 2016 Other details and inclusion/exclusion criteria not provided	Semen samples were collected according to current WHO recommen-dations Concentration (µg/mL of ejaculate) of molybdenum and 19 other metals determined using ICP-MS LOD/LOQ not reported	Spermogram analysis according to WHO manual using the recommended normal ranges - ejaculate volume - absolute and relative sperm count - sperm motility - sperm vitality Statistical analysis:	Inverse association ($p<0.05$) between Mo concentration and seminal liquid volume Mo concentration was not associated with - sperm count - sperm concentration - sperm motility - sperm vitality	Analyses not adjusted for potential confounders	Reporting is poor with some essential information and all tables missing

		Spearman's rank correlation coefficients between Mo concentration and semen quality parameters.	
Zeng et al., 2015 [74] Cross-sectional study Wuhan, China 394 men blindly and randomly selected from 2090 men April 2011 – May 2012 Inclusion criteria: - Presenting at reproductive center for semen analysis Exclusion criteria: - Azoospermia, orchiditis, epididymitis, vesiculitis, vasectomy, undescended testicle, injury of testis, hernia repair complicated by testicular atrophy, and endocrine disease (e.g., diabetes, thyroid, or adrenal disorders).	Single spot urine samples Concentration of Mo and 12 other metals determined using ICP-MS LODs ranged from 0.001 to 0.29 µg/L Expressed as creatinine-adjusted urinary concentration (µg/g creatinine) Quartiles of Mo concentration Q1: <28.99 Q2: 28.99-41.63 Q3: 41.64-68.46 Q4: >68.46 µg/g creatinine	Semen samples provided by masturbation and analysed according to WHO guidelines: - semen volume - sperm count - sperm concentration (million/mL) - sperm motility (% A+B motile sperm) - sperm normal morphology (%) - sperm abnormal head (%) Statistical analysis: - Multivariable logistic regression analysis for associations between quartiles of creatinine-adjusted urinary Mo concentrations and sperm concentration, motility,	Mo was detected in all urine samples, (median 42, range 8–425 µg/g creatinine) and associated with almost all other metals Sperm concentration <20 million/mL: n=46 Sperm motility <50% motile: n=222 Sperm count <40 million: n=38 Control group with all three parameters ≥reference values: n=169 No associations between quartiles of Mo concentration and below-reference semen quality parameters or morphology

		count, and morphology dichotomized using WHO reference values - All models adjusted for age, abstinence time and smoking status	Mo was not retained in the analytical models including multiple metals
Wang et al., 2016 [75] Cross-sectional study Wuhan, China 1052 men of sub-fertile couples (mean age 32 years) visiting reproductive center for semen analysis March –June 2013 Exclusion criteria: - Azoospermia, self-reported health conditions that may affect male reproductive health or urinary metals excretion, occupational exposure to metals	Two spot urine samples several hours apart (mean: 4.4 ± 3.7 h) Concentration (µg/L) of Mo and 17 other metals determined using ICP-MS LOQ not specified Geometric mean concentrations were calculated from the results of the 2 samples and grouped into quartiles	Serum hormones in blood samples drawn between 08:30 and 11:30 AM (n=511): - oestradiol - follicle stimulating hormone (FSH) - luteinizing hormone (LH) - sex hormone-binding globulin (SHBG) - total testosterone (T) - derived measures: total T/LH ratio, free androgen index, free T Sperm characteristics in semen samples collected after 2-7 days of abstinence: - spermatozoa apoptosis (n=460), reported as % necrotic, % apoptotic, and %	Mo was detected in all urine samples - First sample: median 68, IQR 44-106 µg/L - Second sample: median 67, IQR 36-103 µg/L No associations between quartiles of average Mo concentration and levels of serum reproductive hormones Total T/LH ratio inversely associated with quartiles of average Mo concentration (FDR-adjusted p for trend 0.02), also when simultaneously adjusted for confounders and multiple metals:

	viable – DNA integrity by neutral comet assay (n=516): tail DNA %, tail length and tail distributed moment Statistical analysis: - Associations between quartiles of Mo level (averaged over 2 samples) and markers of male reproductive health outcomes assessed using multivariable linear regression models, adjusted for: age, BMI, smoking status, daily cigarette consumption, and urinary creatinine - False-discovery rate (FDR) correction to account for multiple testing - Restricted cubic spline functions to assess dose-response associations with reference values set to median for associations found in multivariable analysis	<25th: 0.00 (Reference) 25th–50th: −5.6% (−19%, 6.2%) 50th–75th: −8.9% (−25%, 5.1%) >75th: −16% (−34%, −1.0%) P for trend 0.03 Dose-response association between average Mo concentration and total T/LH ratio: - P for overall association: 0.01 - P for non-linear association 0.20 No associations between quartiles of average Mo concentration and apoptosis markers or sperm DNA integrity parameters Analyses with Mo concentrations from separate urine samples did not yield different findings

Zhou et al., 2016 [76] Cross-sectional study Wuhan, China 207 men of subfertile couples visiting reproductive center for semen analysis March – June 2012 Exclusion criteria: - Azoospermia, orchiditis, epididymitis, vesiculitis, vasectomy, undescended testicle, varicocele, injury of testis, and hernia repair complicated by testicular atrophy	Single spot urine samples Concentration of Mo and 12 other metals determined using ICP-MS LOD 0.004 µg/L Expressed as creatinine-adjusted urinary concentration (µg/g creatinine) Grouped into quartiles Q1: <26.77 Q2: 26.77-38.94 Q3: 38.95-58.60 Q4: >58.60	Semen samples were collected after abstinence time ranging from ≤2 to ≥6 days Neutral comet assay to assess sperm DNA damage: - percent DNA tail - tail length - tail distributed moment Statistical analysis: Multivariable linear regression analysis to assess dose-response relationships between quartiles of creatinine-adjusted urinary metal levels and comet assay parameters Adjustment for: - age - BMI - smoking status - abstinence time	Mo detected in all urine samples (median 39 µg/g creatinine) No associations between quartiles of Mo concentration and comet assay parameters.		
Branch et al. (2021) [142] Cross-sectional study	Urine sample collected upon completion of baseline interview	Semen samples were collected twice at home: (1) day after enrolment	Mo was detected in all urine samples		Unclear how data from the two separate semen samples per

413 men (aged 19-51 years) participating in Longitudinal Investigation of Fertility and the Environment (LIFE) Study Michigan and Texas, USA 2005–2009 Inclusion criteria: - men from couples who were discontinuing contraception in an attempt to achieve pregnancy - at least 18 years of age - married or in a committed relationship - able to communicate in English or Spanish - without physician-diagnosed infertility	Concentration (µg/L) of Mo and 14 other metal(loid)s determined using ICP-MS LOD not reported Expressed as creatinine-adjusted urinary concentration (µg per g creatinine)	interview; (2) approximately 1 month later (mean abstinence time 4 days) Semen analysis included quantification of 7 endpoints: - total sperm count ($\times 10^6$/ejaculate) - semen volume (mL) - sperm concentration ($\times 10^6$/mL) - next day motility (%) - traditional morphology (%) - DNA fragmentation index (%) - high DNA stainability (%) Statistical analysis: [i] penalized LASSO regression models to identify and select metal(loid)s most likely to be predictive of each semen quality endpoint and potential confounders [ii] unpenalized multivariable linear regression models with metal(loid)s and confounders selected in the LASSO regression only	(median=46.65 µg/L or 38.36 µg/g creatinine) Analysis [i]: Mo concentration was only selected for inclusion for sperm motility Analysis [ii]: no association between Mo and motility: beta=0.07 (95%CI −0.3 to 0.44), adjusted for race/ ethnicity, study site and urinary levels of As, Cr, Pb, Tl, Sn, W, and U Analysis [iii]: no associations between Mo concentration and any of the semen endpoints - total sperm count: −0.03 (−0.63 to 0.58) - semen volume: +0.03 (−0.08 to 0.14) - sperm concentration: −0.13 (−0.54 to 0.28) - next day motility: +0.21 (−0.12 to 0.54) - traditional morphology: −0.30 (−2.35 to 1.74)		participant were treated in analyses

		- Potential confounders: abstinence time; age; race/ethnicity; alcohol consumption; BMI; education; household income; fathered previous pregnancy; urinary creatinine; current smoking status (serum cotinine); study site [iii] multivariable linear regression models for Mo concentration and all semen endpoints, adjusted for confounders but NOT for other metal(loid)s	- DNA fragmentation index: +0.73 (−0.75 to 2.20) - high DNA stainability: −0.04 (−0.81 to 0.72)

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1 Table A2 Summary of epidemiological cross-sectional studies relevant for female sexual function and fertility

2

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Syrkasheva et al., 2021 [78] Cross-sectional study Moscow, Russia 30 women (aged 18-39 years), residents of Moscow for the last 5 years Couples who applied for assisted reproductive technology (ART) 2017 to 2018 Inclusion criteria: - no contraindications for ART - normal karyotype of both spouses - absence of severe male factor - BMI 19–25 kg/m2 Exclusion criteria: - use of donor gametes or surrogacy - obtaining ≤3 oocytes on day of transvaginal ovarian puncture	Blood samples taken on day of transvaginal puncture Concentration (µg/L) of molybdenum and 30 other elements determined using ICP-MS LOD/LOQ not reported	ART included ovarian stimulation with gonadotropin releasing hormone antagonists, transvaginal ovarian puncture, and in vitro oocyte fertilization blood sample 14 days after embryo transfer with measurements of: - Human chorionic gonadotropin (β-hCG) - antimullerian hormone (AMH) - free thyroxine (T4free) Clinical pregnancy defined as registration of the embryo's heartbeat 5 weeks after transfer. Other outcome variables:	Mo was detected in all blood samples (median 0.705µg/L) Mo concentration increased with increasing age (r=0.384; p=0.036) No associations between Mo concentration and any of the health outcome parameters	Analyses not adjusted for potential confounders	Results of analyses for Mo not provided; in tables a selection of metals with statistically significant results only

	- number of previous pregnancies - gynaecological diseases: endometriosis, myoma, inflammatory diseases of pelvic organs - primary or secondary infertility and duration - features of the ovarian stimulation protocol: duration of stimulation and total dose of gonadotropins - parameters of oogenesis and early embryogenesis, e.g. number of blastocysts obtained Statistical analysis: Pearson's correlation coefficients and Mann-Whitney U test for Mo concentration and continuous health outcomes Chi-square test for categorical outcomes	

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Fertility – animal data

Table A3: Summary of animal studies on effects of molybdenum compounds on fertility

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
Jeter et al. (1954)	Long-Evans rats (N=4-8/sex/group)	Fertility and development study Animals were exposed for 20 weeks. Animals were allowed to mate from eleven weeks onwards. Experimental design related to fertility study: Histological sections were made of the testes. Assessment of number of litters, litter size and weight. In order to determine the effect on the oestrus cycle, 4 females were fed a ration containing 700 ppm and vaginal smears were made over a 5-week period.	Approximately <0.04 mg (0 ppm), 0.9 mg (20 ppm), 3.5 mg (80 ppm), 6.2 mg (140 ppm) molybdenum/kg bw/day approximately 31 mg molybdenum/kg bw/day for 700 ppm dosed animals Test item: disodium molybdate dihydrate in diets containing 5 ppm copper (normal copper content 1.8 ppm)	Decrease in average weight gain of male rats at 20, 80 or 140 ppm molybdenum (140 g, 147 g, 80 g, respectively. Controls 176 g)), and of females at 80 and 140 ppm molybdenum (105 g and 85 g respectively. Controls 128 g) over the first eleven weeks. Depigmentation of the hair and alopecia were observed in some rats fed 20, 80 or 140 ppm molybdenum.	At 80 and 140 ppm molybdenum, males were successful in mating in one of four cases. Mating of the treated males with untreated females did not result in pregnancy. In contrary, mating of females given 80 or 140 ppm molybdenum with untreated males resulted in pregnancy rates of 100%. Histopathologic examination of the testes of males treated with 80 and 140 ppm molybdenum revealed degeneration of the seminiferous tubules.	

					Mature virgin female rats showed irregular oestrus cycles after receiving the rations containing 700 ppm molybdenum for 10 days, whereas controls had a normal oestrus cycle.
Schroeder et al., 1971 [80]	Five pairs of Charles River CD mice	Breeding study over three generations. F0 animals were allowed to breed freely for 6 months. Animals were at random selected from the first three litters to form the F1 and allowed to breed to form the F2 (period not indicated). Animals of the first two F2 litters were selected to form the F3-generation. Age at first litter, interval between litters, number of litters and total pups, litter size, male-female ratios, number of deaths,	+/- 1.7 – 2 mg molybdenum/kg bw/day (10 mg/L molybdenum in deionized drinking water and 0.45 ppm molybdenum in diet) Test item: molybdate (cation unknown)	No mortality was observed in the F0-generation and F1-generation. One maternal death in the F2-generation (versus 1 in controls). Four maternal deaths in the F3-generation.	F0: Age at first litter and interval between litters were similar to control values. No other data on this generation are available. F1: In the F1-generation, no differences between treatment group and controls were reported for number of litters, litter size and number of runts. Fifteen of the 238 F1 mice died early (not further specified). In the selected animals of the F1- generation, one

	failures to breed and number of runts were assessed.		female died. The interval between the litters was increased (43 versus 28 days in controls), but the age at first litter was not affected. F2: The number of F2 litters, litter size, and dead young were similar to controls. Five of the 26 litters were found dead compared to 0 out of 23 in controls. In the selected F2, four maternal deaths were reported, and the age at first litter was increased from 62 to 79 days. No effect on interval between litters was found. The number of litters and litter size were decreased in treated animals. F3: Four litters in the F3 were found dead. The numbers of runts (11

					versus 0 in controls) and dead young (34 versus 1 in controls) were increased.
Fungwe et al., 1990[81]	Weanling female Sprague-Dawley rats (N=21/group)	Weanling female Sprague-Dawley rats (N=21/group) were given drinking water with 0, 5, 10, 50 and 100 mg/L molybdenum* as sodium molybdate dihydrate for 6 weeks. Thereafter, rats were exposed during three oestrus cycles before being mated with untreated males (N=15/group) or sacrificed (N=6/group). The mated females remained exposed during gestation until necropsy on day 21.	0, 5, 10, 50 and 100 mg/L molybdenum[a] as sodium molybdate dihydrate	During the first six weeks of the study, no effects on body weight became apparent.	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 hrs in a majority of the 10 -100 mg Mo supplemented animals. Pregnancy rate was not affected by treatment.
Howell et al., 1993	Mature female (n=8/dose) and male (12 in total) Hartley albino guinea pigs,	The effect of ammonium molybdate (AM) and thiomolybdate (TM, presumably ammonium tetrathiomolybdate) in drinking water on the trace element status, reproductive capacity of guinea pigs was studied.	130 µmol TM/L, 261 µmol TM/L, or 261 µmol AM/L[b] 212 µmol Cu/kg, fed ad libitum on a diet Test item: ammonium molybdate or thiomolybdate	Subcutaneous oedema was found only in 1/8 and 4/8 female adult guinea pigs of the high TM dose groups, C and E. Upon X-ray examination, an ossified ridge in the mid shaft	All adult females had oestrus cycles and conception rates were reported to be unaffected. (Number of pregnant animals: group A 7, group B 4, group C 6, group D 6, group E 8, group F 6.)

	weighing around 500-600 g	When each female entered the third oestrus cycle, males were introduced twice a day. Females of dose groups A (control), B (261 µmol AM/L), C (261 µmol TM/L), and D (130 µmol TM/L) received molybdenum compounds from the first day of the oestrus cycle onwards, whereas treatment of group E (261 µmol TM/L) and F (130 µmol TM/L) females was started immediately after mating .		region of the femur was observed in the TM-dose groups (frequencies: 3/5, 0/7, 4/5, and 1/7 for groups C, D, E, and F, respectively), but not in the AM-treated animals nor in any of the pups.		
Study report, 2016[83]	Sprague Dawley rats, males and females. N = 10/sex/dose	Dose-range finding study for the two-generation study by Murray et al. (2019). Study duration: Males: 10 weeks before cohabitation, during the cohabitation period, and continued through to the day before euthanasia Females: 10 weeks before cohabitation, during the cohabitation when male diets and water were used, gestation,	0, 3, 20 and 40 mg molybdenum/kg bw/day in diet or via drinking water. Test item: Sodium molybdate dihydrate Purity: >99%	*Drinking water* Reduced bodyweight with 11.6% (day 71) and 10.6% (day 99) and ~14% reduced body weight gain (day 1 to 99) in males at 40 mg Mo/kg bw/day Dose related increase in molybdenum levels in serum, liver and kidney of parental animals, indicating absorption of molybdenum. Levels were generally higher	*Drinking water* Pregnancy in 10, 9, 9 and 6 rats in the 0, 3, 20 and 40 molybdenum /kg bw/day exposure groups. Pregnancy rate of 6/10 was outside of the historical control average for pregnancy at the testing facility. Reduction in the number of live born pups and an increase in still born pups in a single litter.	GLP compliance: yes Only summary available, from REACH registration dossier. Original study report not available. Therefore, the number of live and still born pups could not be deduced. Results primarily presented in a qualitative manner.

	and lactation periods until Day 21 of lactation (rats that delivered a litter) or Day 25 of presumed gestation (rats that did not deliver a litter). Examination: sperm parameters, litter observations, postmortem examinations of parental animals and offspring, reproductive indices and offspring viability indices.	from diet than from drinking water. Dose related increase in molybdenum levels in serum and tissue (at termination on PND 22). Levels were generally higher from diet than from drinking water. *Diet* Reduced bodyweight in males (11.9% day 99) and in females (9.9% day 71, ~10% on GD 7 and ~12% on GD 10 and GD 20) and reduced body weight gain in males (~14% day 1 to 99) and in females (17.6% days 0 to 20 and 18.8% days 7 to 20) at 40 mg Mo/kg bw/day Dose related increase in molybdenum levels in serum, liver and kidney of parental animals, indicating absorption of molybdenum. Levels	*Diet* No effects on reproduction.

				were generally higher from diet than from drinking water.		
Murray et al., 2019[143]	Sprague Dawley rats, males and females. N = 24/sex/dose	OECD TG 416 (two generation reproductive toxicity study) P-males: exposure for at least 10 weeks before cohabitation, during the cohabitation, and continuing through to the day of euthanasia (total 147-151 days) P-females: for at least 10 weeks before cohabitation, during the cohabitation, gestation, littering and post-partum periods (lactation period) and continuing through to the day of euthanasia (total 156-158 days). F1: during lactation, 10 weeks pre-mating, cohabitation, and continued through the day of euthanasia. Effect parameters as described in OECD TG 416.	0, 5, 17, or 40 mg molybdenum (Mo)/kg bw/day in drinking water. Additional group: 40 mg molybdenum /kg bw/day via diet. Test item: Sodium molybdate dihydrate Purity: 99%	*Drinking water* No effect on body weights or body weight gain. *Diet* 5.9% (day 71) and 8.6% (day 143) decrease in body weight in males at 40 mg/kg bw/day compared to controls 4% (day 71, n.s.), 6-7% (GD 7, 10 and 14) and 22% (GD 0 to 7) decrease in body weight in females at 40 mg/kg bw/day Decreased food consumption (males only) and water consumption at 40 mg/kg bw/day.	*Drinking water* Increase in average number of primordial follicles in the left ovary of parental females at 17 mg/kg bw/day, and in the right, left, and combined ovaries in the F1 generation at 17 and 40 mg/kg bw/day. All values were within the historical control range. *Diet* Increase in average number of primordial follicles in the left ovary of parental females and in the right, left, and combined ovaries in the F1 generation. All values were within the historical control range. Increased percent of sperm with no head in the	Well-performed study; GLP.

					parental generation compared to the control value.
Murray et al. 2023	Weanling Sprague Dawley rats, females, n=24/dose	Female exposure for 8 weeks prior to mating, through cohabitation and pregnancy until Gestation Day 21. The untreated male breeder rats were directly exposed the same concentrations of SMD in the water and the AIN-93 G diet as were the females during the cohabitation phase only. Evaluation of maternal body weights, food consumption, oestrous cycles, elemental analysis of serum Ovarian/uterine examination: weight, number and distribution of corpora lutea, implantation sites, placentae and early and late resorptions. Foetal evaluation after necropsy dams on GD 21: weight, sex, external examination. (no visceral and skeletal examinations)	0, 20, or 40 mg molybdenum (Mo)/kg bw/day in drinking water With marginal copper (6.2 ppm) in diet Test item: Sodium molybdate dihydrate Purity: 99.9%	Body weight gain was generally marginally higher than controls, with occasional statistical significance at 20 mg Mo/kg/day (GD 9–12 $p \leq 0.01$), and 40 mg Mo/kg/day (DS 58–61, GD 0–3, 3–6; $p \leq 0.01$) Throughout the gestation period, water consumption was significantly ($p \leq 0.05$ or $p \leq 0.01$) higher than controls on most occasions after GD 2–3 and ranged from 111% to 142% of controls at 20 mg Mo/kg bw/day and 104–145% of controls at 40 mg Mo/kg bw/day.	No statistically significant effect on mating or fertility parameters

[a] Assuming a mean water intake of 50 to 125 mL/kg bw/day for SD rats, the units in mg/L correspond to a daily intake of approximately 0.25-0.625 mg/kg bw (5 mg/L), 0.5-1.25 mg/kg bw (10 mg/L), 2.5-6.25 mg/kg bw (50 mg/L), and 5.0-12.5 mg/kg bw (100 mg/L).
[b] Assuming a mean water intake of 100 to 170 mL/kg bw/day for guinea pigs, the units in µmol/L correspond to a daily intake of approximately 8.70 mg AM/kg bw (261 µmol/L), 11.55 mg TM/kg bw (261 µmol/L), and 5.75 mg TM/kg bw (130 µmol/L). Based on Mol Wt. of AM divided by atomic mass of Mo, (B) 8.70 mg AM/kg bw is equivalent to 4.71 mg Mo/kg bw. Based on Mol Wt. of TM divided by atomic mass of Mo, (C, E) 11.55 mg TM/kg bw is equivalent to 4.26 mg Mo/kg bw, (D, F) 5.75 mg TM/kg bw is equivalent to 2.12 mg Mo/kg bw

Table A4: Summary of animal studies on effects on reproductive organs from repeated dose studies

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on reproductive organs or reproduction	Remarks
National Toxicology Program, 1997[86]	Fischer 344 rats (N=10/sex/dose) And B6C3F1 mice (10/sex/dose)	Rats and mice received molybdenum trioxide by inhalation for 6.5 hour per day, 5 days per week for thirteen weeks	0, 10, 30, and 100 mg molybdenum trioxide/m^3 (in aerosol) by inhalation Test item: molybdenum trioxide	Body and organ weights, perma-chemical and haematological parameters, and histopathological findings were not different from the control values.	In exposed male rats, sperm counts were unaffected. In addition, no statistically significant effect was observed on the concentration of epididymal spermatozoa. At 10, 30 and 100 mg/m^3, rats showed slightly decreased absolute epididymis weights (0.48 g, 0.49 g and 0.47 g, respectively) compared to unexposed rats (0.50 g). However, these effects were not statistically significant.	

					In exposed mice, absolute cauda epididymis weight was slightly increased (0.025 g versus 0.018 g in controls) at 10 mg/m^3, and absolute testis weight was slightly decreased (0.10 g versus 0.12 g in controls) at 100 mg/m^3. However, these effects were not statistically significant. No statistically significant effects were observed on sperm count, and on the concentration and motility of epididymal spermatozoa in any of the treatment groups.
Pandey, R. and Singh, S.P., 2002	adult male Druckery rats (body weight at start of experiment averaged 120 g)	Sodium molybdate was administered to groups of 10 male Druckery rats for 5 days/week for 60 days Body weights were measured at the start and end of the experiment. And the rats were sacrificed in order to evaluate organ weights of the testes, epididymis, seminal vesicles and prostate glands. Also molybdenum contents were	0, 10, 30, or 50 mg sodium molybdate per kg bw by gavage.[a] Test item: Sodium molybdate	No effects on body weight or clinical signs that could be related to treatment were observed.	50 mg/kg bw • testis (relative 1.15 +/- 0.03 versus 1.20 +/- 0.03 in controls (mean +/- SE)), epididymis (relative 0.32 +/- 0.02 versus 0.38 +/- 0.01 in controls), seminal vesicles (absolute 0.08 +/- 0.01 g versus 0.18+/- 0.013 g in controls, relative 0.05 +/- 0.008 versus 0.08 +/- 0.006 in controls), and prostate gland weights (absolute 0.05 +/-

		determined in the testis, epididymis, and seminal vesicle and these tissues were used for histopathological and biochemical assessment (for testicular enzymes sorbitol dehydrogenase, lactate dehydrogenase and g-glutamyl transpeptidase). Spermatozoa were counted and sperm motility and morphology were assessed. Distribution of molybdenum in reproductive tissues were determined in highest dosed group animals	0.01 g versus 0.11 +/- 0.01 g in controls, relative 0.03 +/- 0.005 versus 0.05 +/- 0.006 in controls) were statistically significantly decreased, and an accumulation of molybdenum was seen in these organs. • Sperm motility was 49.1 +/- 1.3% versus 86.0 +/- 2.3% in controls. Total sperm count 5.0 +/- 0.05 * 10^7 versus 8.0 +/- 0.17* 10^7 in controls (oddly the authors did not indicate this difference as statistically significant) • Reduced concentrations of testicular enzymes • Elevated molybdenum concentrations in the epididymis, seminal vesicle and prostate gland. 30 mg/kg bw • epididymis weight (absolute 0.50+/-0.02 g, relative 0.30 +/- 0.02 versus absolute 0.81 +/- 0.01 g, relative 0.38 +/- 0.01 in

					controls) absolute weight of seminal vesicles (0.09 +/- 0.012 g versus 0.18 +/- 0.013 in controls), and relative weight of the prostate gland (0.04 +/- 0.002 versus 0.05 +/- 0.006 in controls) were statistically significantly decreased. • Sperm motility was 65.0 +/- 1.2% versus 86.0 +/- 2.3% in controls. Total sperm count 6.0 +/- 0.07 * 10^7 versus 8.0 +/- 0.17* 10^7 in controls At both concentrations, degeneration of the seminiferous tubules in the testis was observed.	
International Molybdenum Association (IMOA), 2011[95, 144]	Sprague-Dawley CD rats (5 animals/sex/ group)	28-day study, non-guideline Animals (5 animals/sex/ group) were given sodium molybdate dihydrate by gavage (once daily) or in their diet (ad libitum), for 28 consecutive days. At the end of the treatment, all animals were killed and postmortem examinations, including	0, 4 or 20 mg molybdenum/kg bw/day. Also one group of animals received the compound by gavage twice daily (10 mg/kg bw/administration for a total of 20 mg/kg bw/day).	The investigators did not find exposure-related adverse effects on any in-life parameters (survival, body and organ weights, food consumption). Furthermore, microscopic	Regarding the 28-day study, histopathology did not reveal abnormalities in the kidneys, testes or epididymis.	The Committee emphasizes that adverse effects on male fertility could have occurred after 28 days, because the spermatogenic cycle in rats takes approximately ten weeks. Furthermore, it

		microscopic pathology, were performed.	Test item: Sodium molybdate dihydrate	examinations revealed slight diffuse hyperplasia of the proximal tubules in the kidneys of two female rats fed 60 mg molybdenum/kg bw/day.		is known that, for instance, effects on the seminiferous tubule can develop in the long term. No guideline, study not conducted in compliance with GLP
International Molybdenum Association (IMOA), 2011[95, 144]	Sprague-Dawley CD rats (10 or 20 animals/sex/group)	90-day study, OECD TG 408, including additional parameters, oestrous cycles and sperm analyses, from OECD TG 416. Animals (10 or 20 animals/sex/group) were fed sodium molybdenum dihydrate at doses of 0, 5, 17, and 60 mg molybdenum/kg bw/day, for 91 or 92 days. At the end of the treatment ten animals of each group were killed for post-mortem examinations. The remaining ten animals (in groups administered 0 or 60 mg molybdenum/kg bw/day) were allowed to recover for a further 60 days,	0, 5, 17, and 60 mg molybdenum/kg bw/day Test item: sodium molybdate dihydrate	In males and females, the mean body weight changes from baseline were statistically significantly decreased at the highest dose level A statistically significant decrease in absolute body weight was observed among male animals from the highest dosed group (15.1% less than controls at 60 mg Mo/kg bw/day) These reductions were partially explained by lower food intake.	No molybdenum-related adverse effects were observed on the gonads, oestrous cycles or sperm parameters in any of the exposed groups. The 60 mg Mo/kg bw/day males had a slight but statistically significant decrease of 15% in progressively motile sperm at the Terminal Sacrifice (59.0% versus 69.4% in the control group).	No molybdenum-related adverse effects were observed on the gonads, oestrous cycles or sperm parameters in any of the exposed groups. According to the authors, the difference in progressively motile sperm (59.0% versus 69.4% in the control group) was due to the control group having a value that approached the upper limit for this parameter among historical control groups and was therefore not

	before they were also killed for postmortem examinations.	Furthermore, microscopic examinations revealed slight diffuse hyperplasia of the proximal tubules in the kidneys of two female rats fed 60 mg molybdenum/kg bw/day. One male administered 60 mg Mo/kg bw/day and assigned to the recovery phase, was found dead on Day 47 of the study. There were no macroscopic or microscopic findings to explain the cause of death. In the absence of any other mortality or clinical signs in other test substance treated animals, this single death is considered incidental and unrelated to test substance administration.	considered a test substance-related finding. The Testing Facility's Historical Control value for progressively motile sperm is 59.8% ± 16.2% which closely approximates the 60 mg Mo/kg bw/day value of 59%. All other changes in sperm motility and morphology were considered unrelated to the test substance because they were small in magnitude and values were compatible with normal biological variability.

Zhai et al., 2013 [91]	ICR mice, males. N = 10/dose	Sub-acute study. Male mice received molybdenum in drinking water for 14 days. Sperm parameters, including the epididymis index, sperm motility, sperm count, and morphology were evaluated. Malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels were determined in testes.	0, 12.5, 25, 50, 100, or 200 mg/L, in drinking water, corresponding to 0, 2.5, 5, 10, 20 and 40 mg/kg bw/d[b]. Test item: sodium molybdate dihydrate Purity not mentioned.	Not reported.	Effects on sperm parameters at ≥20 mg/kg bw/day, compared with controls: decrease in epididymis index, decreased sperm motility, decreased sperm concentration and an increased sperm abnormality rate. Effects on sperm parameters at 5 mg/kg bw/day, compared with controls: increase in epididymis index, increased sperm motility, increased sperm concentration and a decreased sperm abnormality rate. Decreased activity of SOD and GPx and increased activity of SOD at ≥20 mg/kg bw/day. Increased SOD and GPx activity at 5 mg/kg bw/day.	Mice were maintained under GLP conditions.
Zhang et al., 2013[92]	ICR mice, females. N=25/dose.	Female mice received molybdenum in the drinking water for 14 days. Relative ovary weight (ovary weight / total body weight = ovary	0, 5, 10, 20, or 40 mg/L, in drinking water, corresponding to 0, 1, 2, 4 and 8 mg/kg bw/d[c].	Not reported.	Increase in number of ovulations at 1 mg/kg bw/day, accompanied by increased GPx activities.	Mice were maintained under GLP conditions. Not clear if other study elements were also performed or

		index). Ovaries were examined by electron microscopy. Oocyte quality was microscopically assessed. Biochemical indicators of oocyte oxidative stress were investigated. It is not clear in how many mice the various parameters were examined.	Test item: Sodium molybdate dihydrate Purity not mentioned.		Decreased ovary index and increased rate of abnormal oocyte morphology at 8 mg/kg bw/day. Morphologically abnormal ovarian mitochondria at 4 and 8 mg/kg bw/day. Changes in antioxidant activity at higher dose levels: Reduced superoxide dismutase activity and increased malondialdehyde contents at 4 and 8 mg/kg bw/day. Decreased GPx activity at 8 mg/kg bw/day.	generated according to GLP.
Wang et al., 2016[93]	Kunming mice, males. N= 20/dose	100-day study, no guideline. Male mice received low and high molybdenum diets (with normal (added as 3 mg/L drinking water) or low cupper levels) and additional molybdenum in drinking water for 100 days. On 50th and 100th day: 10 mice per group were anesthetized after 12h of fasting.	400 mg Mo/L drinking water for 100 days, corresponding to 80 mg/kg bw/day[d]. Test item: Molybdenum compound and purity not specified. Control group was included.	*High molybdenum, 3 mg/L Cu* Decreased body weight on day 14 and day 21 Reduced total protein and albumin levels in serum on day 100 *High molybdenum, low Cu*	*High molybdenum, 3 mg/L Cu* Reduction of sperm density . Degenerated and atrophic germinal cells in the lumina of the tubules. Morphological changes in many spermatogenic cells, including reduced amounts of chromatin, cellular nuclear volume loss, endoplasmic reticulum dilation, and nuclear membrane breakage or	Molybdenum compound not specified. The text was not consistent with the figures, resulting in uncertainty in the data.

	Morphological changes in testicular tissue (haematoxylin and eosin staining and transmission electron microscopy) Analysis of sperm characteristics (however, exact characteristics not specified) Superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and total antioxidant capacity (T-AOC) were analysed in blood serum and homogenized testes.	Decreased body weight on day 28 and day 35 Reduced total protein and albumin levels in serum on days 50 and 100. Increased blood urea nitrogen on day 50	disappearance. Extensive vacuolization and swelling of the mitochondria of the spermatogenic cells and sperms. Decreased superoxide dismutase and total antioxidant capacity in testicular tissue on days 50 and 100 increased malondialdehyde level in testicular tissue on day 100 *High molybdenum, low Cu* Decreased sperm density Degeneration and disorganization of testicular tissues were observed in the germinal cells and tubular epithelium Idem morphological changes in many spermatogenic cells as high molybdenum group increased malondialdehyde level in testicular tissue on days 50 and 100

Khorami et al., 2020[94]	Wistar rats, males. N = 6/dose	30-day testicular toxicity study. Male rats were treated for 30 consecutive days by oral gavage. The right testis was homogenized for biochemical assays (oxidative stress parameters superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione peroxidase (GPX)). The left testis was processed for histopathology. Parameters analysed included: sperm motility, sperm count, sperm viability, sperm abnormalities and sperm membrane integrity.	Controls, 0.05, 0.1, 0.2, and 0.4 mg/kg bw/day[e]. Controls: distilled water. Test item: sodium molybdate Purity: not mentioned.	No data were provided on general toxicity.	No adverse effects on sperm count, sperm viability, sperm morphology, sperm membrane integrity or sperm motility. No effects on oxidative stress. No adverse effects based on histopathological analysis.

[a] Based on Mol Wt. of sodium molybdate divided by atomic mass of molybdenum, 0, 10, 30, and 50 mg sodium molybdate/kg bw is equivalent to 0, 4.7, 14.0, 23.3 mg molybdenum/kg bw
[b] Conversion from mg/L to mg/kg bw/day is based on an assumed water consumption of 5 ml for a mouse weighing 25 g, based on the default values as reported in ECHA guidance R.8, version 2.1, Table 8-17.

[c] Conversion from mg/L to mg/kg bw/day is based on an assumed water consumption of 5 ml for a mouse weighing 25 g, based on the default values as reported in ECHA guidance R.8, version 2.1, Table 8-17. Based on Mol Wt. of sodium molybdate dihydrate divided by atomic mass of molybdenum, 0, 1, 2, 4, and 8 mg sodium molybdate dihydrate/kg bw is equivalent to 0, 0.4, 0.8, 1.6, 3.2 mg molybdenum/kg bw.

[d] Conversion from mg/L to mg/kg bw/day is based on an assumed water consumption of 5 ml for a mouse weighing 25 g, based on the default values as reported in ECHA guidance R.8, version 2.1, Table 8-17

[e] Based on Mol Wt. of sodium molybdate divided by atomic mass of molybdenum, 0, 0.05, 0.1, 0.2, and 0.4 mg sodium molybdate/kg bw is equivalent to 0, 0.02, 0.05, 0.09, and 0.19 mg molybdenum/kg bw

Development – human data

Table A5. Summary of epidemiological studies on effects of molybdenum on development: prospective cohort studies

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Shirai et al., 2010 [145] Prospective cohort study Tokyo, Japan 78 pregnant women visiting obstetric outpatient hospital clinic, without clinical signs of any diseases 2007-2008	Single spot urine sample at 9-40 gestational weeks, during regular maternal health check-ups. Urinary concentrations of Mo and 9 other metals measured by ICP-MS, creatinine-corrected. LOD 5.9 µg/g creatinine	Birthweight (kg), birth length (cm), and head circumference (cm) of newborns at time of delivery. Statistical analysis: - correlation analysis (Pearson's r) - multivariable linear regression analysis, with adjustment for gestational age, sex of newborn, birth order, maternal BMI (maternal height for analysis of birth length), maternal age, maternal or paternal smoking, and other urinary metal concentrations	Urinary Mo concentrations (µg/g creatinine): GM (GSD) 79.0 (1.72), range 10.3–369 No correlations or associations between urinary Mo concentrations and birthweight, birth length, and head circumference.	Potential residual confounding by e.g. alcohol intake or nutrition Measurement in urine sample only once and at variable stages of pregnancy.	Population: recruitment not clearly described and inclusion and exclusions criteria only globally described.

Vázquez-Salas et al., 2014 [98] Prospective cohort study Morales State, Mexico 147 women of reproductive age and their children randomly selected from 294 eligible women January 2001 - June 2005 Inclusion criteria: - urine sample available in at least one trimester of pregnancy - at least 5 of 7 follow-up visits between 1 and 30 months after birth - singleton pregnancy - birth weight ≥2 kg - mother aged >15 years Exclusion criteria: - maternal history of chronic illness or treated with anticonvulsants - breastfeeding at time of recruitment	Urine samples collected during each trimester of pregnancy Concentration of Mo (µg/L) determined in duplicate using electrothermal atomic absorption spectrometry LOD 0.2 µg/L Mo concentration also expressed as µg/g creatinine	Neurodevelopment: Follow-up visits at 1, 3, 6, 12, 18, 24 and 30 months: - Bailey's scale (psychomotor (PDI) and mental (MDI) indexes) - Anthropometry - History of breastfeeding - Diet information Statistical analysis: - Associations between Mo concentration in each trimester of pregnancy and infant neurodevelopment (PDI and MDI separately) estimated using multivariable generalized mixed effect models with age at follow-up as random effect - Mo concentrations in each trimester assessed as [1] µg/L; [2] µg/L with adjustment for creatinine concentration; and [3] µg/g creatinine - Adjustment for potential confounders (fixed effects): gestational age, parity, maternal age, education, occupation, and IQ, birth weight, type of birth, sex of child, breastfeeding,	Molybdenum detected in all urine samples Median Mo concentration levels: - First trimester: 38.5 µg/L; 48.9 µg/g creat. - Second trimester: 39.6 µg/L; 59.1 µg/g creat. - Third trimester: 37.7 µg/L; 58.6 µg/g creat. Inverse associations between urinary Mo concentration in third trimester and PDI, expressed as change of index by doubling of MO concentration: - [1] -0.49 (95% CI -0.1, 003) - [2] -0.54 (95% CI -1.1, -0.002) - [3] -0.57 (95% CI -1.1, -0.1) Adding blood lead levels during pregnancy to the multivariable models (n=64) did not change the results. No associations between urinary Mo concentrations in first and second trimester and PDI No associations between urinary Mo concentrations in any trimester and MDI	Overadjustment by one or more of the potential confounders cannot be ruled out.

- diagnosis of severe cerebral atrophy, early birth, neonatal death, congenital hypothyroidism, cleft lip and palate, or perinatal asphyxia	quality of home environment at 6 months of age, and prenatal DDE exposure

Bloom et al., 2015 including corrigendum [99, 100] Prospective cohort study Michigan (4 counties) and Texas (12 counties), USA Study period: 2005 - 2009 235 couples with singleton pregnancies from 501 couples planning pregnancy, participating in the Longitudinal Investigation of Fertility and the Environment (LIFE) study Couples recruited from general population with presumed exposure to persistent organic pollutants Inclusion criteria: - committed heterosexual relationship - women aged 18–40 years - men aged ≥18 years - English or Spanish speaking	Spot urine samples were collected from both mothers and fathers before conception Concentrations of Mo (µg/L) and 20 other elements determined using ICP-MS LOD not reported Concentrations of Mo divided into tertiles, separately for mothers and fathers	Baseline questionnaire (administered at home by research nurse) on demographics, health-related behaviours, medical history, and reproductive histories Health parameters studied: - Gestational age (days) - Birth weight (kg) - Birth length (cm) - Head circumference (cm) - Ponderal index (100×birth weight/birth length^3) - Infant sex Statistical analysis - Multivariable linear regression analysis for gestational age, birth weight, birth length, head circumference, and ponderal index as continuous outcomes - Cox-proportional hazards analysis for gestational age - Log-binomial models for infant sex - Adjustment for maternal age, difference between maternal and paternal ages, maternal and paternal smoking, income, race,	Molybdenum detected in all urine samples Mothers (n=215): - 1st tertile 1.86-17.91 µg/L - 2nd tertile 17.91-50.44 µg/L - 3rd tertile 50.44-256.69 µg/L Fathers (n=213): - 1st tertile 5.22-32.30 µg/L - 2nd tertile 32.30-75.62 µg/L - 3rd tertile 75.6-268.822 µg/L No associations between maternal or paternal Mo concentrations before conception and any of the outcome parameters under study Interaction between infant sex and continuous paternal Mo concentration for head circumference: - boys: -0.57 (95% CI -1.11, -0.03) cm - girls: 0.10 (95% CI –0.42, 0.62) cm	Strong point of the study is use of pre-conception exposure, also of fathers Study participants presumed to be at risk of environmental exposure, but most values were relatively low compared to US population

- no use of injectable contraceptive within 12 months - menstrual cycle length of 21–42 days	total serum lipids (a proxy for persistent organic pollutants), and creatinine
Exclusion criteria - couples with sterilized partner or prior infertility diagnosis	- Analyses were repeated using log-transformed continuous Mo concentrations instead of tertiles to detect linear trends

Ashrap et al., 2020 [146] Prospective cohort study Mexico City, Mexico 997 women recruited in first trimester of pregnancy and their sons 1997 - 2004 Inclusion criteria: not planning to leave the area within 5 years; no history of infertility, diabetes, or psychosis; not consuming alcoholic beverages daily during pregnancy; no addiction to illegal drugs; no diagnosis of a high-risk pregnancy; being pregnant with singleton.	Women: interview-based questionnaires at 3 visits during pregnancy, spot urine samples in 3rd trimester (n=212). Boys: spot urine samples in early adolescence (n=118, 8-14 years). Urinary Mo and 13 other metal(loid) concentrations measured with ICP-MS. LOD 2.9 µg/L	Early adolescence fasting serum samples analysed for: oestradiol, testosterone, inhibin B, sex hormone-binding globulin (SHBG) and dehydroepiandrosterone sulphate (DHEA-S) Physical examination (sexual maturation) in early and late adolescence: Tanner staging of genital and pubic hair development and testicular volume assessed by two trained paediatricians. Statistical analyses: - multivariable linear regression to assess associations between urinary Mo and hormone levels - generalized estimating equation (GEE) to explore associations between urinary Mo and sexual maturation parameters - all analyses were adjusted for child age, BMI z-score. and urinary specific gravity (SG) as	Prenatal urinary Mo concentrations (µg/L): GM 19.5, median 25.7, IQR 12.7-42.9 Peripubertal children's Mo concentrations (µg/L): GM 46.6, median 50.2, IQR 33.7-67.1 Differences (%) in peripubertal hormone concentrations associated with IQR increase in Mo concentration <u>in utero</u>: - Oestradiol -1.1 (-10.0, 8.7) - Testosterone 51.3 (19.1, 92.4), remained statistically significant after correction for multiple testing -Inhibin B 2.9 (-6.9, 13.8) - SHBG -1.1 (-10.3, 9.0); - DHEA-S -0.1 (-12.5, 14.2) No differences (%) in peripubertal hormone concentrations associated with IQR increase in Mo concentration <u>peripubertal</u>: - Oestradiol -0.9 (-10.0, 9.3) - Testosterone -18.1 (-38.2, 8.6) - Inhibin B -2.1 (-12.3, 9.3) - SHBG 3.5 (-7.0, 15.1) - DHEA-S 5.5 (-8.8, 22.2)	Results not adjusted for exposure to other metal(loid)s.

	measure of urinary dilution, but not for other metal(loid)s	No associations between in utero and peripubertal Mo concentrations and (changes in) genital development, pubic hair development, and testicular volume. Sensitivity analyses: In prepubertal boys (n=94), difference (%) in testosterone concentrations associated with IQR increase in Mo concentration in utero 119.4 (31.5, 266). Similar estimates with and without adjustment for BMI or household SES.

Howe et al., 2020 [102] Prospective cohort study Los Angeles, CA, USA 2015 - 2019 Population: 262 participants in the Maternal and Developmental Risks from Environmental and Social Stressors (MADRES) study, i.e. pregnant women recruited at one of four prenatal care providers in LA, mainly lower-income Hispanic populations Exclusion criteria: pregnancy ≥20 wks of gestation at recruitment, <18 years of age, hiv positive, physical, mental or cognitive disability, multiple gestation, incarceration, no urine sample at first visit, missing covariate information	Spot urine samples collected during first study visit (median gestational age 13.1 weeks) Concentrations of Mo and 9 other metals measured by ICP-MS	- Birth weight for gestational age and sex, z-scores based on a 2017 US reference (Airs, et al. 2019) - Birth weight measures obtained from medical records; if missing (n=22) based on information from mother - Gestational age estimates using ultrasound or observation at birth (physician's estimate). Statistical analysis: - primary analysis focused on combination of 7 metals, excluding Mo. - secondary exploratory analysis included Mo and all other metals, but excluded 3 participants with unusually low MO concentrations - associations between metal mixture and outcome analysed using Bayesian kernel machine regression Directed acyclic graphs (DAGs) were used to identify potential confounders: recruitment site,	Urinary Mo concentrations (urine specific gravity corrected, µg/L): median 56.8, IQR 42.9-80.7 Posterior inclusion probability for Mo in secondary exploratory analysis was 0.41, which ranked in fifth place of importance. No dose-response relation was observed for Mo concentration and birth weight z-scores.	Only subjects with complete covariate information were included.	Focus of this study on mixtures of metals. Impoverished urban study population, probably above average at risk of exposure and intra-uterine growth retardation

Other publication in the same cohort: Howe et al. (2021), different health outcome and fewer participants	self-reported maternal age, pre-pregnancy BMI, race by ethnicity and birthplace, and smoke exposure during pregnancy, as well as measured pregnancy anaemia and urinary arsenobetaine (as marker of fish consumption).

Kim et al., 2020 [147] Prospective cohort study Boston, MA, USA 2006-2008 390 participants in LIFECODES birth cohort study, i.e. pregnant women planning hospital delivery, enrolled before week 15 of pregnancy and participating in up to four study visits. Inclusion criteria: pregnancy resulting in preterm birth (n=130, almost all occurrences) or in at term birth (n=352, randomly selected in 3:1 ratio) originally selected for nested case-control study Exclusion criteria: no urine sample from third study visit (=26 weeks of pregnancy) available.	Concentrations of Mo and 16 other metals in urine samples, collected at median 26 range 20-32) weeks of pregnancy, measured with ICP-MS and corrected for urine specific gravity LOD 0.30 pbb Demographics, lifestyle factors, medical and pregnancy history obtained by questionnaire.	Parameters of foetal growth, measured by ultrasound at weeks 26 (median, range 20-32) and 35 (median, range 30-40), following guidelines of ACOG: - Abdominal circumference (mm) - Head circumference (mm) - Femur length (mm) - Estimated foetal weight (EFW) from these measures, following Hadlock formula; - Z-scores based on gestational age at scan, with all singleton pregnancies in the hospital in 2006-2012 as reference. - Birth weight (g), birth length (cm), and placental weight (g) (in subset). Statistical analysis: - Linear mixed effect models for associations between metals and repeated outcome measures (at 26 weeks, 35 weeks, birth), - Linear regression for associations with birth weight, birth length, and placental weight.	Mo concentrations all above LOD. Mo specific-gravity-corrected concentrations (ppb), weighted for case control design, median (IQR): 51.3 (37.1-69.7). Single metal models adjusted differences in z-scores for repeated measures of foetal growth associated with IQR increase in urinary Mo (β (95% CI)): Femur length 0.15 (-0.05, 0.35) Head circumference -0.05 (-0.22, 0.13) Abdominal circumference -0.02 (-0.21, 0.17) EFW + birth weight 0.02 (-0.14, 0.18) No associations with birth weight, birth length, and placental weight in adjusted linear regression analyses for single metals. Multi-metal models adjusted differences in z-scores for repeated measures of foetal growth associated with IQR difference in urinary Mo (β (95% CI)): Femur length 0.30 (0.08, 0.52)	Ultrasounds at weeks 26 and 35 (visits 3 and 4) were taken at participant's request or when abnormality suspected → availability of ultrasound measurements was selective (sampling bias).

	- Co-variables (in all adjusted models): urine specific gravity, maternal age, race/ethnicity, education, pre-pregnancy BMI, type of insurance, self-reported use of alcohol and tobacco, assisted reproduction, gestational age at time of ultrasound, gestational age at delivery (when appropriate), and metal co-exposure (in multi-metal models)	Head circumference 0.16 (-0.04, 0.36) Abdominal circumference 0.11 (-0.11, 0.33) EFW + birth weight 0.12 (-0.07, 0.31) No associations with birth weight, birth length, and placental weight in adjusted linear regression analyses for multi metals.
	- Inverse probability weighting (IPW) to account for case-control selection - Sensitivity analyses (amongst others) on missing data (multiple imputation by chained equation method)	Sensitivity analyses showed similar associations with slightly attenuated effect estimates.

Howe et al., 2021 [104] Prospective cohort study Los Angeles, CA, USA 2015 - 2019 Population: 193 participants in the Maternal and Developmental Risks from Environmental and Social Stressors (MADRES) study, i.e. pregnant women recruited prior to routine anatomy ultrasound scan at one of four prenatal care providers in LA, mainly lower-income Hispanic populations -Exclusion criteria: pregnancy ≥20 wks of gestation at recruitment, <18 years of age, hiv positive, physical, mental or cognitive disability, multiple gestation, incarceration, no urine sample at first visit, missing covariate	Spot urine samples collected during first study visit (median gestational age 12.4 weeks) Concentrations of Mo and 9 other metals measured by ICP-MS	Mid-pregnancy foetal growth measures evaluated at 18-22 weeks (median 20.4) of pregnancy and obtained from medical records: - Abdominal circumference - Head circumference - Biparietal diameter - Femur length - Estimated foetal weight (EFW) EFW was main outcome in statistical analysis. Statistical analysis: - primary analysis focused on combination of 6 metals, including Mo. - secondary exploratory analysis included Mo and all other metals - associations between metal mixture and outcomes analysed using Bayesian kernel machine regression - metals with high-ranking posterior inclusion probabilities further analysed with linear regression models	Urinary Mo concentrations (urine specific gravity corrected, µg/L): median 57.4, IQR 44.3-81.1 Posterior inclusion probabilities for Mo ranked highest in both primary (0.631) and secondary analysis (0.485) for EFW. Setting other metals to their median, an increase in Mo concentration from the 25th to 75th percentile was associated with a 0.114 (95% CI: 0.019, 0.247) SD higher EFW, equivalent to a ~7.4 g higher EFW. Visually, this association was attenuated at higher levels of barium (Ba), but the p value for interaction between Mo and Ba was 0.22 in linear regression analysis. Based on posterior inclusion probabilities, Mo consistently contributed most to the associations with all other foetal growth parameters as well. An interquartile change in Mo concentration was associated with a 0.30 (95% CI: 0.05, 0.56) SD difference in head circumference. Visual attenuation of	Only subjects with complete covariate information were included.	Focus of this study on mixtures of metals. Impoverished urban study population, probably above average at risk of exposure and intra-uterine growth retardation.

information, low Mo concentration (n=2). Other publication in the same cohort: Howe et al. (2020), different health outcome and more participants	Directed acyclic graphs (DAGs) were used to identify potential confounders: recruitment site, gestational age at ultrasound, self-reported maternal age, pre-pregnancy BMI, race by ethnicity and birthplace, education, infant sex, parity, prenatal vitamin use, and smoke exposure during pregnancy, as well as measured urinary arsenobetaine (as marker of fish consumption).	the positive association of Mo with head circumference at higher levels of Ba was confirmed in linear regression analysis (p for interaction 0.03). .

Karakis et al., 2021 [148] Prospective cohort study Negev desert, Israel Dec 2011 – Mar 2013 plus 5.4-6.9 years of follow-up 111 mothers and their singleton newborns of Bedouin-Arab origin, recruited at obstetrics emergency department of Soroka University Medical Center (SUMC) Inclusion criteria: - member of 'Clalit' health maintenance organization (HMO) - ≥18 years of age - urine sample collected prior to birth - newborn survived birth hospitalization	Urine samples collected just prior to delivery Concentration (ppb) of Mo and 24 other metals determined using ICP-MS LOQ=0.01 ppb	Medical records prepared by local hospital and/or HMO personnel provided information on: - preterm delivery - small-for-gestational age (SGA) - congenital malformations - behavioural/developmental disorders - other disorders during follow-up Statistical analysis: - metal concentrations ranked into quintiles - associations with health outcomes analysed using Poisson regression analysis, adjusted for maternal age, parity, newborn gender, and preterm birth	Mo concentration in urine (pbb): GM 7.23 (95% CI: 3.86, 13.55) Associations between Mo concentration in quintiles and clinical outcome: adjusted Relative Risks (p-values) - preterm delivery: 1.32 (0.129) - congenital malformations: 0.89 (0.655) - behavioural or developmental disorders: 1.86 (0.016) No results reported for SGA	Explorative analysis Relatively small size and hence limited statistical power

McKeating et al., 2021 [149] Prospective cohort study Adelaide, Australia 117 pregnant women May 2009 - July 2013 Inclusion criteria: - Pregnant women attending first antenatal visit - aged >18 years	Urine and blood plasma samples were collected at 18-week visits at the clinic Concentrations of Mo (µg/L in plasma and ng/L in urine) and 36 other elements were determined using ICP-MS LOD/LOQ not reported	Neonatal data were collected at delivery and resulted in: - 13 preterm birth (<37 weeks) - 10 small for gestational age (SGA / lowest 10%) - 87 healthy infants (controls) Statistical analysis - Associations between element levels and neonatal outcomes: - One-way ANOVA followed by post-hoc pairwise comparisons	Plasma concentration (mean±SD in µg/L) - Preterm birth: 0.71±0.15 - Small for gestational age: 0.72±0.13 - Controls: 0.89±0.32 Urine concentration (mean±SD in ng/L) - Preterm birth: 4.12±2.1 - Small for gestational age: 3.34±0.98 - Controls: 4.66±2.34 No statistically significant differences between infants with adverse outcomes and controls	Plasma samples were not available for all participants Small sample sized of adverse neonatal outcomes

Tung et al. (2022) [107] Prospective cohort study Rhode Island, US. 192 mother-infant pairs recruited from hospital in Rhode Island into Rhode Island Child Health Study (RICHS) Oversampling of term infants born large for gestational age and small for gestational age 2010 – 2011 Inclusion criteria: - mothers ≥18 years without life-threatening medical complications - infants born free of life-threatening medical complications or congenital or chromosomal abnormalities	Placenta parenchyma tissue biopsied approximately 2 cm from cord insertion site and free of maternal decidua within 2 hours of delivery. Placental levels of Mo and 23 other trace elements analysed with ICP-MS.	Newborn neurobehavioral performance assessed with NICU Network Neurobehavioral Scale (NNNS), administered by certified psychometrists 24 – 72 hours after birth. NNNS score patterns categorized into 5 profiles of which profile 5 indicates most atypical neurobehavioral performance. Statistical analysis: - associations between individual metals and NNNS profiles (profile 5 vs. other profiles) assessed with multivariable logistic regression models, adjusted for infant sex, maternal age, maternal race, pre-pregnancy BMI, and education status during pregnancy - quantile g-computation for association between mixture of 8 metals (including Mo) and NNNS profile 5 vs. other profiles	Placental Mo concentration (ng/g): mean 6.76, median 6.58, IQR 5.85-7.42 Adjusted odds ratios for NNNS profile 5 per doubling of placental Mo concentration approximately 2 with large 95% CI including unity. Mo played a very small role in association between metal mixture and NNNS profiles	As the RICHS cohort focused on aberrant foetal growth, over half of the included infants were born small or large for gestational age, but analyses were not adjusted for birth weight category.

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1 *Table A6. Summary of epidemiological studies on effects of molybdenum on development: case-control studies*

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Yan et al., 2017 [108] Case-control study Shanxi Province and Hebei Province, China 2003-2007 Cases: 191 women with a pregnancy complicated by a neural tube defect (live births, stillbirths, and pregnancy terminations). Controls: 261 women who delivered full-term healthy infants in same birthing hospital, loosely matched on county/city of residence and last menstrual period.	Unpainted maternal hair sections, grown from 1 month before to 2 months after conception (assuming hair growth rate of 1 cm per month) collected Mo and 8 other essential trace metals measured with ICP-MS. LOD not reported	NTD subtypes: anencephaly (n=85), spina bifida (n=79), encephalocele (n=24), and unspecified (n=3). Statistical analysis: Comparison of hair Mo concentrations between groups: Mann-Whitney U test Unconditional multivariable logistic regression with dichotomized Mo concentrations based on median in controls as cut-off value and correction for covariables. Dose-response analysis by estimating adjusted ORs for quartiles of Mo concentrations based on quartiles in controls . Co-variables: maternal age, occupation, education, gravidity, history of previous birth defects, fever or flu during early	Mo concentrations in maternal hair (median, IQR in ng/mg hair) and adjusted odds ratios (95% CI): Total NTDs (n=191) cases: 0.071 (0.062-0.084) controls: 0.075 (0.063-0.088) P-value 0.032 Adjusted OR: 0.64 (0.42-0.98) Anencephaly (n=85) Cases: 0.071 (0.062-0.084) Controls: 0.075 (0.063-0.088) P-value 0.070 Adjusted OR: 0.51 (0.28-0.94) Spina bifida (n=79) Cases: 0.071 (0.063-0.082) Controls: 0.075 (0.063-0.088) P-value 0.039 Adjusted OR: 0.54 (0.31-0.94) Encephalocele (n=24) cases: 0.074 (0.062-0.091)	Differences between cases and controls for several co-variables, that were adjusted for in the analyses. No multivariable analyses with co-exposure to other metals. To maximize the sample size, matched pairs were separated for the analysis with unconditional logistic regression.	Unexpected inverse associations observed between Mo concentrations in maternal hair and NTDs in offspring.

	pregnancy, alcohol consumption, periconceptional folate supplementation, active or passive smoking during periconceptional period (collected by face-to-face interview within first week after the end of pregnancy).	controls: 0.075 (0.063-0.088) P-value 0.786 Adjusted OR: 0.74 (0.29-1.94) Dose-response analysis showed decreasing trends in adjusted ORs (95% CI) with increasing quartiles of Mo concentrations for total NTD, anencephaly, and spina bifida. Total NTDs (n=191) Q1: 1.00 (ref) Q2: 1.08 (0.65-1.79) Q3: 0.95 (0.57-1.61) Q4: 0.58 (0.33-1.02) Anencephaly (n=85) Q1: 1.00 Q2: 0.95 (0.43-2.09) Q3: 0.66 (0.29-1.48) Q4: 0.35 (0.14-0.87) Spina bifida (n=79) Q1: 1.00 Q2: 1.15 (0.55-2.42) Q3: 0.79 (0.36-1.72) Q4: 0.38 (0.16-0.92) Encephalocele (n=24)

			Q1: 1.00 Q2: 0.77 (0.19-3.17) Q3: 0.36 (0.07-1.71) Q4: 1.02 (0.27-3.89)
Deyssenroth et al., 2018 [109] Case-control study Rhode Island, US 2009-2013 195 mother-infant pairs selected from Rhode Island Child Health Study (RICHS) Inclusion criteria: - mothers ≥18 years - infants without congenital or chromosomal abnormalities - complete molecular profile (placental RNA-Seq) and metal exposure data available Cases: infants small for gestational age (SGA, <10% percentile)	Maternal toenail clippings following hospital discharge (average time to collection 2.8 months (range, 0.3–7.1 months) postpartum) Concentration of Mo and 18 other metals analysed using ICP-MS.	Anthropometrics from structured reviews of medical records. Statistical analyses: Associations between Mo concentration and SGA status assessed using logistic regression. Metal mixture indices associated with SGA status derived with weighted quantile sum (WQS) regression; robustness of the major drivers of SGA status assessed with Bayesian kernel machine regression (BKMR). All regression models adjusted for infant gender, maternal ethnicity, maternal BMI, and maternal smoking status during pregnancy.	Mean (SD; min-max) Mo toenail concentrations (µg/g dry weight): 0.018 (0.032; 0.002-0.366). Adjusted OR (95% CI) for association between log unit increase in Mo concentration and SGA (approximated from figure): 0.8 (0.4-1.2) WQS multi-metal index weight for Mo near zero, indicating low variable importance in driving the association of metal mixtures with SGA, corroborated in BKMR analysis.

Controls: infants born appropriate for gestational age (AGA), matched to cases on gender, gestational age, and maternal age				
Hou et al., 2019 [110] Nested case-control study Guangxi Province, China 2015-2016 Participants in Guangxi Birth Cohort Study Cases: 246 women with low birth weight children Controls: 409 women with normal birth weight children, matched on maternal age, infant gender, gestational age at sample collection, and enrolment hospital in a 1:2 ratio Exclusion criteria: multiple pregnancy, gender	Concentrations of Mo and 21 other metals measured with ICP-MS in serum samples collected during prenatal examination.	Health outcome: birth weight - low birth weight (cases) < 2500 g - normal birth weight: 2500-4000 g Measures obtained from medical records database. Statistical analysis: Single metals associated with LBW using conditional logistic regression with quartiles of Mo concentration, adjusted for pre-pregnancy BMI, alcohol consumption pre-pregnancy, passive smoking during pregnancy, gravidity, and parity. Restricted cubic splines (RCS) to assess dose-response of Mo with LBW, adjusted for pre-pregnancy BMI.	Serum Mo concentration (µg/L), median (IQR): - cases: 1.18 (0.90; 1.64) - controls: 1.07 (0.89; 1.37) Adjusted OR (95%CI) per quartile of Mo (based on distribution among controls): - Q1: (≤ 0.90 µg/L): 0.75 (0.48-1.16) - Q2: (0.91-1.07 µg/L): 0.48 (0.30-0.77) - Q3: (1.08-1.37 µg/L): 0.71 (0.45-1.10) - Q4: (>1.37 µg/L): 1.00 (ref) - P-trend: 0.018. Stratification according to gender, gestational age (≤ vs. >13 weeks), maternal age (≤ vs. >28 y) resulted in similar ORs.	Pre-pregnant BMI distribution different (p=0.001) between cases and controls, with more cases being underweight (BMI <18.5), but adjusted for in the analysis. Gestational age at delivery lower in cases than controls (35.5 versus 39.1 y, p<0.001), as expected with LBW.

information missing, serum sample missing.	Multi-metal exposure analysed by elastic net regression followed by conditional logistic regression, adjusted for same potential confounders.	RCS analysis: no dose-response relationship between serum Mo concentration and LBW. In multi-metal analysis, 15 metals including Mo were associated with LBW: OR 5.41 (2.81-9.40)

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1 *Table A7. Summary of epidemiological studies on effects of molybdenum on development: cross-sectional studies*

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Pi et al., 2019 [111] Cross-sectional study Shanxi Province, China January 2003 through December 2016 Rural population Cases: 103 newborns and terminated foetuses with orofacial clefts (OFC). Controls: 206 newborns without congenital malformations, randomly selected from 509 non-malformed newborns with available placental tissues, matched to cases by mother's residence, date of last menstrual period, and newborn sex.	Approximately 6 g of tissue from foetal portion of placenta sampled after delivery or pregnancy termination Placental concentrations of Mo and 5 other metals assessed with ICP-MS. Analysis staff blinded to case or control status.	Diagnosis of OFC by county healthcare workers through physical examinations and prenatal ultrasound scans. Face-to-face interviews by local health care workers based on structured questionnaire to assess information on potential confounders. Statistical analyses: Differences between cases and controls in median Mo concentrations tested with Mann-Whitney U-test. Association between above/below median Mo concentrations and OFC estimated with unconditional logistic regression, adjusted for maternal age, BMI, farming occupation, influenza or fever, passive smoking, alcohol drinking during periconceptional	Placental Mo concentrations (ng/g dry weight; median (IQR)) in cases and controls: 35.9 (31.7–41.8) and 32.1 (27.3–37.0), respectively; P<0.001. Associations between above (≥33.6 ng/g) vs. below (<33.6 ng/g) median Mo concentrations and risk of OFCs: crude OR 2.20 (1.36, 3.58), adjusted OR 1.42 (0.78, 2.59). ORs for tertiles of Mo concentration: <30.1 ng/g: reference value 30.1-36.6 ng/g: crude OR 2.30 (1.22, 4.32), adjusted OR 1.98 (0.95, 4.13) ≥36.6 ng/g: crude OR 3.14 (1.68, 5.87), adjusted OR 1.46 (0.67, 3.21) P for trend 0.001 (crude) and 0.354 (adjusted).	Potential overadjustment by gestational age, which should not be included as a confounder No adjustment for co-exposure to other metals	Analyses not adjusted by folic acid use because folic acid use was similar (ca. 50%) in the case and control groups.

		period, gestational age, and history of pregnancy affected by birth defects. Dose-response analysis comparing ORs for tertiles of Mo concentration			
Troisi et al., 2019 [112] Cross-sectional study Three hospitals in Southern Italy Study period January 2011 to December 2013 Cases: 111 pregnant women diagnosed with foetal malformations (n=67) or foetal chromosomal abnormalities (n=44), recruited during second trimester termination of pregnancy. Exclusion criteria: age > 40 y, twin pregnancy, women committed to carrying the pregnancy to term, TORCH (Toxoplasma, Rosolia, Citomegalovirus,	Fasting blood samples collected in 2nd trimester immediately before termination of pregnancy and before any drug administration (cases) or during the routine scan (controls). Serum concentrations of Mo and 43 other metal(loid)s determined with ICP-QMS. Clinical history and demographics assessed by questionnaire and complete obstetric visit at enrolment.	Foetal malformations or chromosomal abnormalities assessed with ultrasound examination or karyotype, confirmed by postmortem autopsy by expert pathologist or after paediatric examination. Statistical analysis: Differences between cases and controls tested with independent t-test with Bonferroni correction. Comparison of metal distribution between the two case groups and controls using Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA).	No differences in natural logarithm (mean ± SD) of serum Mo concentrations (µg/L) between controls (-3.09 ± 1.10), CNS group (-2.48 ± 0.57), and other malformations group (-4.12 ± 1.10), p>0.0006. PCA: no aggregation of subjects. PLS-DA: Mo was 9th among the 15 metal(loids) most important in distinguishing the CNS group from the other malformations group	Analyses were not adjusted for potential confounders.	Low number of cases in the CNS group. No information provided on the types of malformations.

Herpes) complex infection, or CNS defects with a known genetic cause. Subdivision into two groups: all CNS malformations with unknown aetiology (n=17) and all other malformations or chromosomal abnormalities (n=94). Controls: 90 women with normally developed foetuses at the same week of pregnancy, recruited during second trimester routine anomaly scan.					
Ovayolu et al., 2020 [113] Cross-sectional study Gaziantep, Turkey November 2017 - July 2018 Cases: 36 women with foetuses with neural tube defects (NTDs)	Amniotic fluid collected during amniocentesis in gestational weeks (mean (SD)): - cases 21.6 (6.6) - controls 19.6 (2.4); P=0.096. Concentrations of Mo and 13 other metals determined by ICP-MS. LOD not reported.	Diagnosis of NTD with ultrasonographic examinations in pregnancy weeks 16-37. Statistical analyses: Differences in Mo concentrations between cases and controls tested with Student's t test. Co-variables assessed, but not matched or adjusted for: maternal age, parity, gravidity,	Mo concentration (µg/L; mean (SD)): - cases 1.11 (1.06); - controls 2.47 (1.92); P<0.001	Cases were younger than controls (27.1 vs 31.3 years, p=0.014) and less frequently had a history of abortion (0.2 vs 0.5, p=0.036).	No multivariable analysis with adjustment for potential confounders or co-exposure to other metals.

Controls: 39 women with unaffected foetuses, matched for maternal BMI and gestational weeks, who underwent amniocentesis because of age-related risk or increased risk in triple test. Exclusion criteria: age <18 years, pregnancies conceived through artificial reproductive techniques, previous pregnancy affected by NTD, chronic diseases, drug use, non-use of folic acid in early weeks of pregnancy, and obstetric complications.		previous births, abortion, frequency of sea food consumption, presence of dental amalgam, and smoking/passive smoking status.		
Yin et al., 2020 [114] Cross-sectional study Shanxi Province, China January 2003 through December 2016 Rural population	Approximately 6 g of tissue from foetal portion of placenta sampled after delivery or pregnancy termination Placental concentrations of Mo and 5 other metals assessed with ICP-MS.	Diagnosis of NTD by county healthcare workers through physical examinations and prenatal ultrasound scans. Face-to-face interviews by local health care workers based on structured questionnaire to assess information on potential confounders.	Placental Mo concentrations (ng/g-dry weight; median (IQR)) in cases and controls: 41.3 (32.8–51.2) and 32.8 (26.8–39.7), respectively;; P<0.001. Similar concentration patterns for anencephaly and spina bifida cases separately. Associations between above (≥35.7 ng/g) vs. below (<35.7 ng/g) median	Placental Mo concentrations may not reflect concentrations during the critical period of neural tube development, because tissue sampling occurred mostly in 2nd or 3rd trimester.

Cases: 408 newborns and terminated foetuses with neural tube defects (NTD) Controls: 593 foetuses or newborns without structural malformations found by foetal ultrasound scan or identified at birth or at pregnancy termination from the same hospital, matched to cases by mother's residence and date of last menstrual period, including controls selected for orofacial cleft cases (see Pi et al., 2019). Exclusion criteria: cases and controls with insufficient placental tissue or incomplete key information.	Analysis staff blinded to case or control status.	Statistical analyses: Differences in Mo concentrations between cases and controls tested with Mann–Whitney U test. Association above/below median or for quartiles of Mo concentrations and NTD risk estimated with multivariable logistic regression, adjusted for gestational age at delivery/pregnancy termination, maternal occupation, maternal education, parity, history of birth defects, fever or flu during early pregnancy, periconceptional folic acid supplementation, maternal passive smoking.. Bayesian kernel machine regression (BKMR) model used to quantify and visualize effects of overall metal exposure and individual components (including Mo) within the context of overall joint exposure, adjusted for the same potential confounders.	Mo concentrations and risk of NTDs: crude OR 3.07 (2.36–3.99); adjusted OR 3.73 (2.74–5.07). Similar results for anencephaly and spina bifida cases separately. OR (95% CI) per quartile Mo concentrations (ng/g): <28.5: reference value 28.5–35.7: crude 1.27 (0.86–1.89); adjusted 1.38 (0.88–2.15) 35.7–44.1: 1.88 (1.28–2.76); adjusted 2.20 (1.42–3.40) ≥ 44.1: crude 6.58 (4.45–9.73); adjusted 9.84 (6.18–15.67). P for trend for crude and adjusted ORs <0.001 Similar results for anencephaly and spina bifida cases separately. No effects of Mo in BKMR model in which all other metals were also included. Sensitivity analyses to investigate potential residual confounding because of correlation between gestational age and Mo concentrations, as cases were	Potential overadjustment by gestational age, which should not be included as a confounder

			mostly electively terminated pregnancies at earlier gestational stage: multivariable analyses repeated in subset of gestational age-matched cases and controls and after exclusion of the gestational age-matched subset resulted in similar results as overall analysis.		
Yin et al., 2020 [115] Cross-sectional study Beijing, Shandong, and Shanxi), China October 2010 - January 2019. Cases: 130 women with foetuses or newborns with orofacial clefts (OFCs): - 76 cleft lip with cleft palate (CLP) - 44 cleft lip only (CLO) - 10 cleft palate only (CPO) 11 cases were complicated with other system malformations (syndromic).	Venous maternal blood samples collected during pregnancy or after birth. Concentrations of Mo and 5 other essential trace elements in serum analysed by ICP-MS.	OFCs confirmed at delivery or elective termination of pregnancy after prenatal diagnosis of malformation. Statistical analysis: - Difference in median Mo concentrations between cases and controls tested with Mann-Whitney U test. - Associations between Mo concentrations and risk for OFCs examined using multilevel mixed-effects logistic regression (to adjust for heterogeneity in Mo by region). - Joint effects of metal co-exposure analysed by Bayesian	Median (IQR) Mo concentrations (ng/mL) differed between cases and controls (p<0.01): Controls: 2.816 (2.392–3.496) Total OFCs: 2.378 (1.757–2.938) CLP: 2.413 (1.835–2.970) CLO: 2.327 (1.727–2.930) Adjusted ORs (95% CI) for tertiles of Mo concentrations with T1 as reference: Total OFCs T2: 0.37 (0.20–0.66) T3: 0.28 (0.15–0.54) P for trend < 0.01 CLP T2: 0.42 (0.20–0.89) T3: 0.35 (0.15–0.80)	No adjustments for maternal age, occupation, or history of birth defects because these variables did not differ statistically significantly between cases and controls, although the history of birth defects differed (6.2% in cases vs. 1.9% in controls).	Number of gestational weeks at blood sample collection differed substantially between cases (31.5% <28 weeks) and controls (78.1% ≥37 weeks). Population partly overlaps with that of Pi, 2019.

Controls: 260 women with non-malformed foetuses or infants. matched on province or city and first day of last menstruation (±4 months)		kernel machine regression (BKMR). - All multivariable models adjusted for gestational weeks, maternal education, flu or fever, periconceptional folic acid supplementation, parity, and passive smoking.	P for trend 0.009 CLO T2: 0.32 (0.14–0.77) T3: 0.27 (0.11–0.67) P for trend 0.004 Similar associations after adjustment for sample collection period and exclusion of 11 cases of syndromic OFCs. Multi-metal analysis (BKMR): Mo serum concentrations inversely associated with risk of OFCs.		
Gomez Roig et al., 2021 [116] Cross-sectional study 167 mother-infant pairs Pregnant women (3rd trimester) were recruited from 2 maternal-foetal and neonatal medicine clinics Barcelona, Spain Period: not reported Inclusion criteria: -healthy pregnant women - aged ≥18 years	After delivery, a full-thickness section (0.5–1 cm x 3–4 cm thick) of a peripheral site of the placenta was taken Concentration (µg/L) of Molybdenum determined using ICP-OES (inductively coupled plasma optical emission spectrophotometry) Limit of detection was 0.0013 mg/kg	Prenatal ultrasound examinations (with colour Doppler imaging) at the beginning of the third trimester Estimated foetal weight centiles were calculated using local reference curves - SGA: small foetuses <10th percentile - AGA: normally grown foetuses >10th percentile Placental function was assessed by measuring the following foetal-maternal parameters:	Mo detected in >80% of the placenta samples Mo concentration: mean 0.01 (SD 0.03); median 0.01 (P25−P75 0.01−0.02) mg/kg Placental Mo concentration and foetal weight: - AGA n=96, Mo mean 0.01 (SD 0.01) mg/kg - SGA n=71, Mo mean 0.01 (SD 0.04) mg/kg Student’s t-test, p for difference 0.89	Potential confounding cannot be excluded since no covariables were included in initial (and for Mo only) analyses	Exposure was determined later (after delivery) than health outcome (at third trimester) or at the same time (pulsatility index arteries) Analyses for Mo were not adjusted for potential confounders Dichotomisation of foetal weight (and artery PI) is probably not optimal for

- with a singleton pregnancy - planning delivery at either of the 2 clinics Exclusion criteria: - multiple gestations - no gestational checkups - no available placental samples	In total 22 chemical elements were determined	- Uterine artery Pulsatility Index (PI) - Umbilical artery PI - Middle cerebral artery PI Statistical analysis: - groups (SGA vs. AGA and dichotomised artery PI) were compared using Student's t-test - A multivariable logistic regression model with the most significant variables in the simple analyses was estimated using a forward variable selection method based on Likelihood Ratio (considered for each of the elements)	Placental Mo concentration and artery PI: - Abnormal uterine artery Doppler (PI>P95) Yes: n=34, mean Mo 0.01 (SD 0.01) mg/kg No: n=120, mean Mo 0.01 (SD 0.04) mg/kg p>0.05 - Abnormal umbilical artery Doppler (PI>P95) Yes: n=8, mean Mo 0.01 (SD 0.01) mg/kg No: n=148, mean Mo 0.01 (SD 0.03) mg/kg p>0.05 - Abnormal middle cerebral artery Doppler (PI<P5) Yes: n=8, mean Mo 0.01 (SD 0.01) mg/kg No: n=148, mean Mo 0.01 (SD 0.03) mg/kg p>0.05 Mo was not selected for multivariable logistic regression analyses	analyses in terms of statistical power 71 out of 167 foetuses (43%) were SGA (<P10), while foetal weight was not part of the selection criteria. In Methods is stated that Mo concentrations were log-transformed but in Tables comparisons between groups are presented as mean with SD
Tian et al., 2021 [117] Cross-sectional study Shanxi province, China	Fasting and non-fasting blood samples: in cases taken during pregnancy,	Diagnosis of NTD by county healthcare workers through	Median (IQR) Mo concentrations (ng/mL): Controls 2.66 (2.03-3.27)	Number of gestational weeks (at which most of the blood samples

2003-2016 Cases: 273 women with NTD-affected pregnancies Controls: 477 women with non-malformed foetuses or newborns who delivered at the same birthing hospital, matched on last menstrual period (±4 weeks).	at termination of pregnancy, or at delivery; in controls initially taken at delivery, but in later study years at similar gestational age (±4 weeks) as cases. Blood serum concentrations of Mo and 9 other metals analysed by ICP-MS. Sociodemographic characteristics and lifestyle collected via face-to-face interviews with structured questionnaire.	physical examinations and prenatal ultrasound scans. NTD subtypes: 97 anencephaly, 127 spina bifida, 29 encephalocele, and 20 NTDs with other malformations Statistical analysis: - Difference in median Mo concentrations between cases and controls tested with Mann-Whitney U test. - Associations between Mo concentrations and NTDs examined using multilevel mixed-effects logistic regression (to adjust for heterogeneity in Mo by region and calendar year) - Joint effects of metal co-exposure analysed by Bayesian kernel machine regression (BKMR). All multivariable models adjusted for maternal age, BMI, education, gestational weeks, sex of the foetus,	All NTD cases 2.51 (1.43-3.07), P=0.002 Anencephaly 2.50 (1.31-3.02), P=0.03 Spina bifida 3.12 (1.31-4.47), P=0.02 Multilevel mixed-effects logistic regression adjusted ORs (95% CI) for tertiles of Mo concentrations with T1 as reference: All NTD: T2: 0.48 (0.26-0.90) T3: 0.54 (0.29-1.00) Anencephaly: T2: 1.04 (0.41-2.65) T3: 0.45 (0.17-1.18) Spina bifida: T2: 0.41 (0.20-0.86) T3: 0.52 (0.54-1.05) Multi-metal analysis (BKMR): ORs for IQR increase in Mo concentration 0.89 (0.83-0.96), 0.87 (0.80-0.94),	were collected) differed substantially between cases (62.3% <28 weeks) and controls (90.7% ≥37 weeks). Population partly overlaps with that of Yin, 2019.

		periconceptional folic acid use, and maternal flu or fever.	and 0.85 (0.78-0.93) when remaining nine elements were set at 75th, 50th, and 25th percentiles, respectively.	
Zhao et al., 2021 [119] Cross-sectional study Hangzhou, China January – December 2016 220 pregnant women during second trimester Inclusion criteria: - singleton foetus in 22-28 weeks of gestation at enrolment - willing to deliver in study hospital, participate in face-to-face interviews, complete ultrasound examinations, and provide urine sample at prenatal care visits in second trimester	Spot urine samples collected at 24.9 ± 0.8 weeks Concentrations (µg/L) of Mo and copper (Cu) determined using ICP-MS LOD 0.031 µg/L	Prenatal ultrasound scanning in 22-26 weeks of gestation. Foetal biometric parameters: - abdominal circumference - biparietal diameter - femur length - head circumference - estimated foetal weight (calculated using Hadlock's formula) Birth weight and infant sex retrieved from medical records Structured questionnaires used to collect information on potential confounders Statistical analysis: - Multivariable linear regression models to estimate associations between Mo concentration and foetal biometric parameters and birth weight, adjusted for maternal age, education, household income, parity, pre-pregnancy BMI, second hand	All samples showed Mo concentration >LOD Median concentration (IQR) was: - 50.3 (30.6−76.0) µg/L (unadjusted) - 61.5 (47.0−87.8) µg/g creatinine (adjusted) Associations between creatinine-adjusted Mo concentration and foetal growth parameters as beta (95% CI): - abdominal circumference (cm): −0.34 (−0.63 to −0.04) - biparietal diameter (cm): +0.02 (−0.06 to +0.09) - femur length (cm): 0.00 (−0.06 to 0.06) - head circumference (cm): −0.01 (−0.26 to 0.23) - estimated foetal weight (g): −18.2 (−40.5 to 4.2) No association between Mo concentration and birth weight (g): +6.8 (−103.6 to 117.2)	No other metals were taken into account.

	smoke in pregnancy, gestational age at ultrasound scanning, and foetal sex - Potential effect modification by urinary copper level explored by stratification on median Cu level	In pregnant women with Cu levels below the median value, associations between Mo concentration and abdominal circumference (-0.55, -1.13 to 0.04) and estimated foetal weight (-42.3, -87.4 to 2.8) were stronger than in women with Cu levels from the median upwards (-0.08, -0.57 to 0.42 and -3.8, -40.2 to 32.6, respectively), with p-values for interaction of 0.340 and 0.222.

Development – animal data

Table A8: Summary of animal studies on effects of molybdenum on development

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
Jeter et al. (1954)	Long-Evans rats (N=4-8/sex/group)	Fertility and development study Animals were exposed for 20 weeks. Animals were allowed to mate from eleven weeks onwards.	Approximately <0.04, 0.9, 3.5, 6.2 mg molybdenum/kg bw/day Test item: disodium molybdate dihydrate in diets containing 5 ppm copper (normal copper content 1.8 ppm)	The growth rates of male rats at 20, 80 or 140 ppm molybdenum, and of females at 80 and 140 ppm molybdenum, were statistically significantly decreased over the first eleven weeks. Depigmentation of the hair and alopecia were observed in some rats fed 20, 80 or 140 ppm molybdenum.	No effect on average birth weight (entire litter) 4.77 g (20 ppm), 4.72 g (80 ppm), 5.07 g (140 ppm) versus 5.21 g in controls. Number of dead pups at birth was 1 at 80 ppm and 11 at 140 ppm versus 0 in controls. Number of dying pups before 21 days was 13 in 20 ppm group, 6 in 80 ppm group and 9 in 140 ppm group, versus 7 in controls. The average pup weight at 21 days was 29.3 g in 20 ppm group, 28.3 g in 80 ppm group and 23.8 g in 140 ppm group versus 32.7 g in controls.	No statistics

Schroeder et al., 1971[80]	Five pairs of Charles River CD mice	Schroeder et al. (1971) exposed five pairs of Charles River CD mice to 10 mg/L molybdenum (as molybdate; cation unknown) in deionized drinking water for up to six months, while the diet contained 0.45 ppm molybdenum. Animals were allowed to breed freely during this period. Animals were at random selected from the first three litters to form the F1, and allowed to breed to form the F2 (period not indicated). Animals of the first two F2 litters were selected to form the F3-generation.	10 mg/L molybdenum in deionized drinking water, while the diet contained 0.45 ppm molybdenum[a]. Test item: molybdate (cation unknown)	Not reported.	F0: No mortality was observed in the F0-generation. Age at first litter and interval between litters were similar to control values. No other data on this generation are available. F1: In the F1-generation, no differences between treatment group and controls were reported for number of litters, litter size and number of runts. Fifteen of the 238 F1 mice died early (not further specified). In the selected animals of the F1- generation, one female died. The interval between the litters was increased (43 versus 28 days in controls), but the age at first litter was not affected. F2: The number of F2 litters, litter size, and dead young were similar to controls. Five of the 26 litters were found dead compared to 0 out of 23	The study was poorly reported.

			in controls. In the selected F2, four maternal deaths were reported, and the age at first litter was increased from 62 to 79 days. No effect on interval between litters was found. The number of litters and litter size were decreased in treated animals. F3: Four litters in the F3 were found dead. The numbers of runts (11 versus 0 in controls) and dead young (34 versus 1 in controls) were increased.

Fungwe et al., 1990[81]	Weanling female Sprague-Dawley rats (N=21/group)	Weanling female Sprague-Dawley rats (N=21/group) were given drinking water with 0, 5, 10, 50 and 100 mg/L molybdenum* as sodium molybdate dihydrate for 6 weeks. Thereafter, rats were exposed during three oestrus cycles before being mated with untreated males (N=15/ group) or sacrificed (N=6/group). The mated females remained exposed during gestation until necropsy on day 21.	0, 5, 10, 50 and 100 mg/L molybdenum[b] as sodium molybdate dihydrate	During the first six weeks of the study, no effects on body weight became apparent.	During gestation, weight gain of the dams was statistically significantly decreased at 10, 50 and 100 mg/L, but these changes were attributed to reduced foetal weights. Gestation weight gain ± SE (g): 0 mg Mo/L: 119.1 ± 4.3 5 mg Mo/L: 119.6 ± 3.1 10 mg Mo/L: 97.7 ± 6.4 50 mg Mo/L: 97.7 ± 7.1 100 mg Mo/L: 93.8 ± 9.4 The number of resorptions was increased in females treated at 10 mg/L and above. Dams with resorbed foetus (n): 0 mg Mo/L: 1/14 5 mg Mo/L: 1/12 10 mg Mo/L: 6/13 50 mg Mo/L: 10/12 100 mg Mo/L: 6/12 Litter size did not differ between treatment groups and controls, but foetal weight and length were decreased at 10, 50 and 100 mg/L.	The subcommittee is of the opinion that the study of Fungwe et al. (1990) gives some indications for effects on the development of the progeny. However, this study is not sufficient for a classification. The remaining studies do not support the findings of Fungwe et al. (1990) as the observed effects were found in the presence of maternal toxicity.

	Average litter size: 0 mg Mo/L: 11.4 ± 0.5 5 mg Mo/L: 11.7 ± 0.6 10 mg Mo/L: 9.4 ± 1.4 50 mg Mo/L: 10.5 ± 1.2 100 mg Mo/L: 9.3 ± 1.3 Growth retardation was observed (less mature hepatic structure, delayed transfer of foetal haemopoiesis to bone marrow, delayed foetal oesophageal development, and myelination in the spinal cord) in the foetuses at 10 mg/L and above. Blood and hepatic enzymes of the dams were affected at 5 mg/L and above. Plasma ceruloplasmin was statistically significantly increased in all gestating dams, but not in dams sacrificed after three oestrus cycles. Hepatic xanthine oxidase/dehydrogenase, and sulphite oxidase, were statistically significantly

					increased in all treated females in the study.
Howell et al., 1993[82]	Mature female (n=8/dose) and male (12 in total) Hartley albino guinea pigs, weighing around 500-600 g	The effect of ammonium molybdate (AM) and thiomolybdate (TM, presumably ammonium tetrathiomolybdate) in drinking water on the trace element status, reproductive capacity of guinea pigs was studied. When each female entered the third oestrus cycle, males were introduced twice a day. Females of dose groups A (control), B (261 µmol AM/L), C (261 µmol TM/L), and D (130 µmol TM/L) received molybdenum compounds from the first day of the oestrus cycle onwards, whereas treatment of group E (261 µmol TM/L) and F (130 µmol TM/L) females was started immediately after mating[c].	212 µmol Cu/kg, fed ad libitum on a diet Test item: ammonium molybdate	Clinical signs observed in several dams of the high TM-dose groups including hair loss, transient diarrhoea, subcutaneous oedema, and mortality before or during pregnancy.	Reduced pregnancy rate in AM-treated females (4 out of 8 animals were pregnant), and an increased 'aborted resorbing' in high TM-dose females (group C). The mean number of pups born alive was reduced in groups B (10), C (3), D (10) and E (0), but not in group F (18) versus controls (21). Pup body weight was slightly decreased at birth in the TM-treated groups C (107.6 g (SE 3.1)), D (108.8 g (SE3.3)), and F (106.1 g (SE 9.7)) versus controls (114.4 g (SE 4.3)) . Six weeks after birth, body weight gain of group C pups (317,4 g (SE 26,1)) was lower as compared to controls (364,2 g (SE 31,3)). No changes in ossified femur was observed in any of the pups.

International Molybdenum Association (IMOA), 2012[120]	Pregnant Sprague-Dawley rats (N=10/ group)	In a dose-range finding study, sodium molybdate dihydrate was administered to pregnant Sprague-Dawley rats from 6 to 20 days of gestation. At gestation day 20, the animals were sacrificed and gross necropsy was performed.	0, 1, 5, 10 and 20 mg molybdenum/kg bw/day in the diet *ad libitum* Test item: sodium molybdate dihydrate	No molybdenum-related general effects (maternal body weight, weight gains, organ weights, clinical observations, feed consumption).	No molybdenum-related developmental toxicity (pre- and post implantation loss, foetal numbers, sex ratio, body weights and or foetal external malformations) was observed.	
International Molybdenum Association (IMOA), 2013[121]	Sprague Dawley rats (N=25/ group)	Dose-range finding study, OECD TG 414 Sodium molybdate dihydrate was administered to pregnant Sprague-Dawley rats from 6 to 20 days of gestation. At gestation day 20, the animals were sacrificed and gross necropsy was performed. This is a follow-up study of International Molybdenum Association (IMOA), 2012.[120] The study was repeated with higher doses.	0, 3, 10, 20 and 40 mg molybdenum/kg bw/day, via the diet Test item: sodium molybdate dihydrate	No treatment-related effects were observed on maternal body weight, weight changes, feed consumption, clinical observations, pregnancy indices or maternal organ weights.	Also no treatment related effects were observed regarding numbers of ovarian corpora lutea, uterine implantation sites and losses, number of foetuses, foetal sex ratios, foetal body weights, foetal external, visceral or skeletal malformations or variations in the foetuses per females.	Chemical analysis by non-GLP facility. The Committee cannot make a final conclusion on the present and the previous study, since a lack of maternal toxicity in combination with a lack of developmental effects may indicate that the chosen exposure levels were too low to induce adverse health effects. In that case, and according to OECD TG 414 (prenatal developmental toxicity study), further investigations are needed.

Murray et al., 2014 [90]	Sprague Dawley rats. N= 25 time-mated females / dose.	OECD TG 414 (prenatal developmental toxicity study). Serum blood, placenta, liver and kidney samples were analysed for molybdenum, copper, zinc, manganese, iron, cobalt and selenium.	0, 3, 10, 20 and 40 mg Mo/kg bw/day, in the diet. The corresponding sodium molybdate dihydrate concentrations in the diet were 0, 100, 338, 675 and 1350 ppm, respectively. Substance: Sodium molybdate dihydrate Purity: 99.9%	No treatment-related adverse effects observed. Dose-dependent increases in molybdenum in serum, placentae, liver and kidneys, accompanied with significant increases in serum and tissue copper levels.	No treatment-related adverse effects on development.	GLP study.

Mohamed et al., 2020 [122]	Mice, strain and 5 mice per dose group.	Molybdenum nanoparticles with a size less than 100 nm were orally administered to pregnant mice from the 1st up to the 17th (group G1, G3) day of pregnancy or from the 9th up to the 17th day (group G2, G4) of pregnancy. Examinations: External (morphological) malformations and skeletal abnormalities.	Oral dose levels of 0, 500 (group G1, G2) or 750 (group G3, G4) mg/kg bw were used. Route of exposure not further specified. Test item: Mo nanoparticles (< 100 nm). Purity: purity was confirmed by X-ray diffraction, no quantitative data.	Decreases in the maternal body weight. DNA damage and elevated expression of levels of p53 gene. Increased expression levels of E-Cad and N-Cad genes that control skeleton development.	Decreased number and length of foetuses. Increased incidence of skeletal abnormalities (reduced ossification and chondrification).	No information on the species and number of animals and lack of data on the test item.

Murray et al., 2019 [143]	Sprague Dawley rats, males and females. N = 24/sex/dose	OECD TG 416 (two generation reproductive toxicity study) P-males: exposure for at least 10 weeks before cohabitation, during the cohabitation, and continuing through to the day of euthanasia (total 147-151 days) P-females: for at least 10 weeks before cohabitation, during the cohabitation, gestation, littering and post-partum periods (lactation period) and continuing through to the day of euthanasia (total 156-158 days). F1: during lactation, 10 weeks pre-mating, cohabitation, and continued through the day of euthanasia. Effect parameters as described in OECD TG 416.	0, 5, 17, or 40 mg molybdenum (Mo)/kg bw/day in drinking water. Additional group: 40 mg Mo/kg bw/day via diet. Test item: Sodium molybdate dihydrate Purity: 99%	*Drinking water* No effect on body weights or body weight gain. *Diet* 5.9% (day 71) and 8.6% (day 143) decrease in body weight in males at 40 mg/kg bw/day compared to controls 4% (day 71, n.s.), 6-7% (GD 7, 10 and 14) and 22% (GD 0 to 7) decrease in body weight in females at 40 mg/kg bw/day Decreased food consumption (males only) and water consumption at 40 mg/kg bw/day.	*Drinking water* No effects on development in F1 and F2 generations *Diet* No effects on development in F1 and F2 generations	Well-performed study; GLP.

Study report, 2020[83]	Sprague-Dawley rats. Non-pregnant females, n=6/group.	14-day toxicity study to test for tolerance of sodium molybdate dihydrate by oral gavage. Preliminary study to the OECD TG 414 study as described below (Study report, 2021). Administration: 14 days (where tolerated) Parameters: viability, clinical signs, body weights, body weight gains, food consumption, bioanalysis, organ weights, and macroscopic observations.	300, 600, and 1000 mg/kg bw/day (equivalent to 120, 240, and 400 mg Mo/kg bw/day, respectively), by oral gavage. Test item: Sodium molybdate Purity: no data	*300 mg/kg bw/day* adverse clinical observations (dehydration, hunched posture, erect fur, cold to touch) Reduced bw (21%) Reduced food consumption (40%) *600 mg/kg bw/day* All euthanized at day 7 (adverse clinical observations, 25% reduction bw, 64% reduction food consumption) *1000 mg/kg bw/day* 4/6 died; 2/6 euthanized	Developmental toxicity not evaluated.

Study report, 2020 [83]	Sprague Dawley rats. N= 10 pregnant females / dose.	Dose range finding study for the OECD TG 414 study described below (Study report, 2021). Administration: GD 6-20, daily treatment. Controls: control group (base diet) and pair-fed control group.	300 and 400 mg/kg bw/day, in the diet. Equivalent to 120 and 160 mg Mo/kg bw/day. Substance: Sodium molybdate dihydrate Purity: no data	Effects in both treatment groups: Reduced maternal body weight (14-23% on GD20) and body weight gain (41-68%) Reduced corrected body weights (terminal body weight at GD21 minus the gravid uterine weight) (16-23%) Reduced food consumption in both groups. Reduced absolute and relative liver weights	Reduced gravid uterine weights at 300 mg/kg bw/day (9%, not statistically significant) and 400 mg kg bw/day (28%) No treatment-related effect as observed in pre- or post-implantation loss, live foetuses per litter, sex ratio or average litter size. Reduced total, male and female foetal weight at 300 and 400 mg /kg bw/day (84%, 72% and 73% respectively) No effect on anogenital distance or external appearance.	Only study summary available, and no absolute data.

Study report, 2021, Aveyard et al. 2023 [123, 124]	Sprague Dawley rats, females. N = 24/group	OECD TG 414 (prenatal developmental toxicity study). Effect parameters as described in OECD TG 414. Additionally to the female rats sacrificed on gestation day 21, 48 female rats were assigned to 2 littering groups (control group and high dose group) of 24 rats which were allowed deliver naturally and raise their young to weaning.	Nominal: 0, 200 and 300 mg/kg bw/day (corresponding to 0, 80 and 120 mg Mo/kg bw/day). Actual dose levels, based on food consumption and body weight: 94.1 and 125.7/128.5 mg molybdenum/kg bw/day. Exposure via diet. Substance: Sodium molybdate dihydrate Purity: no data	Dose-dependent moderate to marked maternal toxicity at both dose levels, including: adverse clinical observations reductions maternal weight gain (27.1% and 49.8% lower than control) reduced food intake (11% and 25%) over the administration period reduced corrected (for uterine content) body weight at gestation Day 21 (12.4 and 23.7% lower than control). Reduced liver weights Test item-related microscopic changes in the kidney: tubular regeneration and mononuclear cell infiltration, more	<u>Caesarean section (GD21)</u> Dose-dependent reductions in foetal weight (~11% and 22% at 200 and 300 mg/kg bw/day) Reduced total placental weight per litter, compared to controls: 11% at 200 mg/kg bw/day (not significant), 24% at 300 mg/kg bw/day. No effect on the incidence of external, visceral and skeletal foetal malformations and variations in the treated animals. <u>Littering animals</u> Lower mean pup weights (combined sexes) in the 300 mg/kg bw/day group, compared to controls, at each interval measured (days 0, 4-preculling, 4-postculling, 7, 14, 18, and 21 postpartum). 19.2% lower pup weight at birth 9.4% lower pup weight at day 21 postpartum Male and female pups similarly affected.	GLP study. Only study summary available, and no absolute data. Reduction in anogenital distances was considered attributable to the marked reduction in foetal weight and marked maternal toxicity, according to the authors of the registration dossier.

	prevalent at 300 mg/kg bw/day Test item-related microscopic changes in the liver: hepatocellular hypertrophy and glycogen accumulation (both dose levels) and karyocytomegaly and vacuolation (300 mg/kg bw/day).	6% reduction in adjusted male foetal anogenital distance at 300 mg/kg/day

Murray et al. 2023	Weanling Sprague Dawley rats, females, n=24/dose	Female exposure for 8 weeks prior to mating, through cohabitation and pregnancy until Gestation Day 21. The untreated male breeder rats were directly exposed the same concentrations of SMD in the water and the AIN-93 G diet as were the females during the cohabitation phase only. Evaluation of maternal body weights, food consumption, oestrous cycles, elemental analysis of serum Ovarian/uterine examination: weight, number and distribution of corpora lutea, implantation sites, placentae and early and late resorptions. foetal evaluation after necropsy dams on GD 21: weight, sex, external examination. (no visceral and skeletal examinations)	0, 20, or 40 mg molybdenum (Mo)/kg bw/day in drinking water With marginal copper (6.2 ppm) in diet Test item: Sodium molybdate dihydrate Purity: 99.9%	Body weight gain was generally marginally higher than controls, with occasional statistical significance at 20 mg Mo/kg/day (GD 9–12 $p \leq 0.01$), and 40 mg Mo/kg/day (DS 58–61, GD 0–3, 3–6; $p \leq 0.01$) Throughout the gestation period, water consumption was significantly ($p \leq 0.05$ or $p \leq 0.01$) higher than controls on most occasions after GD 2–3 and ranged from 111% to 142% of controls at 20 mg Mo/kg bw/day and 104–145% of controls at 40 mg Mo/kg bw/day.	No sodium molybdate dihydrate related effects on resorptions and foetal body weight or foetal malformations or variations

[a] Assuming a mean water intake of 167 to 200 mL/kg bw/day and a food intake of 120 to 150 g/kg bw/day, the total intake of molybdenum per day approximates 1.7 to 2 mg/kg bw.
[b] Assuming a mean water intake of 50 to 125 mL/kg bw/day for SD rats, the units in mg/L correspond to a daily intake of approximately 0.25-0.625 mg/kg bw (5 mg/L), 0.5-1.25 mg/kg bw (10 mg/L), 2.5-6.25 mg/kg bw (50 mg/L), and 5.0-12.5 mg/kg bw (100 mg/L).
[c] Assuming a mean water intake of 100 to 170 mL/kg bw/day for guinea pigs, the units in µmol/L correspond to a daily intake of approximately 8.70 mg AM/kg bw (261 µmol/L), 11.55 mg TM/kg bw (261 µmol/L), and 5.75 mg/kg bw (130 µmol/L).

B Literature search strategy

The Health Council of the Netherlands issued an advice on molybdenum in 2013. That advice was based on (metallic) molybdenum (CAS # 7439-98-7), molybdenum trioxide (CAS # 1313-27-5) and sodium molybdate (CAS # 7631-95-0), the only compounds for which sufficient scientific literature was available at the time. For the present report, a literature search for publications on reproductive toxicity of molybdenum has been performed using various databases from 2012 up to August 2022. Additionally, publications on (toxico)kinetics and monitoring were searched for as well. Below the literature search strategy and its results is presented for the search until April 2021. An update of the search was performed in August 2022.

Embase

Table B1 presents the search terms and the results for the database Embase.

Table B1. Search strategy and result for Embase.

No.	Query	Results
#1	'molybdenum'/exp	17,196
#2	'molybdenum complex'/exp	1,180
#3	'molybd*'	27,836
#4	#1 OR #2 OR #3	27,836
#5	'prenatal exposure'/exp OR 'maternal exposure'/exp OR 'paternal exposure'/exp	28,190
#6	(('prenatal' OR 'maternal' OR 'paternal') NEAR/3 'expos*'):ti,ab	29,085
#7	'reproductive toxicity'/exp OR 'teratogenicity'/exp OR 'developmental toxicity'/exp OR 'ferotoxicity' OR 'embryotoxicity'/exp	36,242
#8	((('repro*' OR 'development*') NEAR/3 'toxic*'):ti,ab) OR 'teratogen*':ti,ab OR 'reprotox*':ti,ab OR 'embryotox*':ti,ab	40,384
#9	#5 OR #6 OR #7 OR #8	101,209
#10	#4 AND #9	96
#11	'fertility'/exp OR 'lactation'/exp OR 'breast milk'/exp OR 'pregnancy'/exp OR 'parameters concerning the fetus, newborn and pregnancy'/exp OR 'infertility'/exp OR 'organogenesis'/exp	1,392,318
#12	'pregnancy outcome*':ti,ab OR 'pregnan*':ti OR 'fertilit*':ti OR 'infertilit*':ti OR 'subfertilit*':ti OR 'fecundit*':ti OR ((('differential'	427,958

	OR 'effect*' OR 'agent*') NEAR/3 'fertilit*'):ti,ab) OR (('breast' NEAR/3 'milk*'):ti,ab) OR (('milk' NEAR/3 'secret*'):ti,ab) OR 'lactation*':ti,ab OR 'organogenes*':ti	
#13	#4 AND (#11 OR #12)	415
#14	'toxicokinetics'/exp OR 'toxicokinetic*':ti,ab	14,029
#15	'bioaccessib*' OR 'bioelut*':ti,ab	3,188
#16	(('environment*' OR 'human' OR 'biologic*') NEAR/3 'exposure monitor*'):ti,ab	123
#17	#4 AND (#14 OR #15 OR #16)	59
#18	'xenobiotic metabolism'/exp OR 'metal metabolism'/mj OR 'metabolism'/mj	231,380
#19	'metabolism':ti OR 'adme':ti,ab OR 'absorption distribution metabolism excretion':ti,ab	238,526
#20	#18 OR #19	441,561
#21	#20 AND [humans]/lim	172,545
#22	'murine'/exp OR 'experimental animal'/exp OR 'animal experiment'/exp OR 'leporidae'/exp OR 'rat':ti,ab OR 'rats':ti,ab OR 'mouse':ti,ab OR 'mice':ti,ab OR 'hamster*':ti,ab OR 'pig*':ti,ab OR 'monkey*':ti,ab OR 'rabbit*':ti,ab	5,465,637
#23	#20 AND #22	142,025
#24	#4 AND (#21 OR #23)	296
#25	#10 OR #13 OR #17 OR #24	779
#26	#25 AND [2012-2021]/py	361

PubMed

Table B2 presents the search terms and the results for the database Pubmed.

Table B2. Search strategy and result for Pubmed.

Search	Search terms	Items found
1	"Molybdenum"[Mesh] OR "molybd*"[tw]	20,451
2	"Prenatal Exposure Delayed Effects"[Mesh] OR "Maternal Exposure"[Mesh] OR "Paternal Exposure"[Mesh] OR "Organogenesis"[Mesh]	154,360

3	"prenatal exposure"[tw] OR "maternal exposure"[tw] OR "paternal exposure"[tw]	41,787
4	"Teratogens"[Mesh] OR "Toxicogenetics"[Mesh]	8,643
5	("reproductive tox*"[tw] OR "developmental toxicity"[tw] OR "fetotoxic*"[tw] OR "teratogen*"[tw] OR "reprotox*"[tw] OR "embryotox*"[tw])	29,793
6	#1 and (#2 or #3 or #4 or #5)	79
7	("Fertility"[Mesh] OR "fertility"[tw] OR "Lactation"[Mesh] OR "Milk, Human"[Mesh] OR "Milk"[Mesh:NoExp] OR "Pregnancy"[Mesh:NoExp] OR "Pregnancy Outcome"[Mesh] OR "infertility"[tw] OR "subfertility"[tw] OR "fecundity"[tw])	1,110,491
8	("pregnancy outcome*"[tw] OR "pregnan*"[ti] OR "fertilit*"[ti] OR "differential fertilit*"[tw] OR "breast milk"[tw] OR "milk secret*"[tw] OR "lactation"[tw] or "infertilit*"[ti] OR "subfertilit*"[ti] OR "fecundit*"[ti] OR "organogenes*"[ti])	382,981
9	#1 and (#7 or #8)	450
10	("Toxicokinetics"[Mesh] OR "Toxicological Phenomena"[Mesh] OR "toxicokinetic*"[tw] OR "bioaccessib*"[tw] OR "bioelut*"[tw])	460,333
11	("exposure monitor*"[tw] AND ("environment*"[tw] OR "human"[tw] OR "biologic*"[tw]))	521
12	#1 and (#10 or #11)	229
13	("Molybdenum/metabolism"[Majr] OR "Metabolism"[Majr:NoExp] OR "metabolism"[ti] OR "adme"[tw] OR "absorption distribution metabolism excretion"[tw])	220,956
14	("rat"[tw] OR "rats"[tw] OR "mouse"[tw] OR "mice"[tw] OR "hamster*"[tw] OR "pig"[tw] OR "pigs"[tw] OR "monkey*"[tw] OR "rabbit*"[tw] OR "human*"[tw] OR "man"[tw] OR "men"[tw] OR "woman"[tw] OR "women"[tw] OR "child*"[tw] OR "infant*"[tw] OR "newborn*"[tw] OR "fetus*"[tw] OR "neonate*"[tw])	23,163,797
15	#1 and #13 and #14	399
16	#6 or #9 or #12 or #15	1,075

17	#16 and 2012:2021[dp]	275

Scopus

The following search terms were used for the database Scopus:

((TITLE-ABS-KEY (*molybd*)) AND ((TITLE-ABS-KEY (('prenatal' OR 'maternal' OR 'paternal') W/3 'expos*')) OR (TITLE-ABS-KEY ((('repro*' OR 'development*') W/3 'toxic*') OR 'teratogen*' OR 'reprotox*' OR 'embryotox*'))OR (TITLE-ABS-KEY ('pregnancy-outcome*' OR 'differential-fertilit*' OR ('breast' W/3 'milk') OR ('milk' W/3 'secret*') OR 'lactation')) OR (TITLE ('pregnan*' OR 'fertilit*' OR 'fecundit*' OR 'infertilit*' OR 'subfertilit*' OR 'organogenes*')))) OR ((TITLE-ABS-KEY ('*molybd*'))AND ((TITLE-ABS-KEY ('toxicokinetic*' OR 'bioaccessib*' OR 'bioelut*' OR (('environment*' OR 'human' OR 'biologic*') W/3 'exposure-monitor*')))OR (TITLE-ABS-KEY ('adme' OR 'absorption-distribution-metabolism-excretion') OR TITLE ('metabolism'))) AND (TITLE-ABS-KEY ('rat' OR 'rats' OR 'mouse' OR 'mice' OR 'hamster*' OR 'pig' OR 'pigs' OR 'monkey*' OR 'rabbit*' OR 'human*' OR 'man' OR 'men' OR 'woman' OR 'women' OR 'child*' OR 'infant*' OR 'newborn*' OR 'fetus*' OR 'neonate*'))) AND PUBYEAR > 2011

This resulted in 108 records.

Toxcenter

Table B3 presents the search terms and the results for the database Toxcenter.

Table B3. Search strategy and result for Toxcenter.

Query	Search terms	Number of records
L1	?MOLYBD?	50,693
L2	(PRENATAL OR MATERNAL OR PATERNAL)(3W)EXPOS?	54,888

L3	(REPRO? OR DEVELOPMENT?)(3W)TOXIC? OR TERATOGEN? OR REPROTO	114,430
L4	PREGNANCY-OUTCOME? OR DIFFERENTIAL FERTILIT? OR BREAST(3W)MILK OR MILK(3W)SECRET? OR LACTATION	43,557
L5	(PREGNAN? OR FERTILIT?)/TI	81,673
L6	TOXICOKINETIC? OR BIOACCESSIB? OR BIOELUT? OR (ENV OR BIOLOGIC?)(3W) EXPOSURE MONITOR?IRONMENT? OR HUMAN	27,036
L7	ADME OR ABSORPTION DISTRIBUTION METABOLISM EXCRETION OR METABOLISM/TI	136,721
L8	L1 AND (L2 OR L3 OR L4 OR L5)	300
L9	L1 AND (L6 OR L7)	375
L10	L9/HUM,ANI	32
L11	L8 OR L10	332
L12	L11 AND 2012-2020/PY	116

ECHA database

The ECHA database was searched for information on the 10 selected molybdenum compounds (see Chapter 3 and Annex A for the selection). These data are included in section 8.

In addition, the database was used to search for registration dossiers of molybdenum-containing substances, e.g. including reaction products and multi-constituent substances, that have information on reproduction toxicity in the registration dossier. These compounds are more complex structures and cannot be used for grouping with molybdenum compounds. Therefore, they are not taken into account for the selection. However, the reproduction toxicity data may be of interest for interpretation of data from molybdenum compounds and are included in Annex B.

Secondary sources

Secondary sources were consulted. These included e.g. IARC, SCOEL, WHO, IPCS, ATSDR, DFG; primarily consulted via echemportal (https://www.echemportal.org). Also RIVM-reports and evaluations and the RIVM-website 'Risico's van stoffen' (https://rvs.rivm.nl/) were consulted.

Overall evaluation of results literature search

The obtained records were evaluated, duplicates were removed, and records were included if considered relevant based on title and abstract. Additionally, publications cited in the selected publications, but not selected during the primary search, were added if considered appropriate.

With respect to human health endpoints evaluated in current report (i.e. reproductive toxicity), this resulted in 19 studies for effects on sexual function and fertility, 27 studies for effects on development and 1 study for effects on or via lactation.