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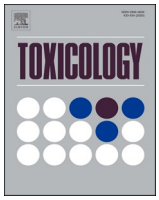

Review

Pulmonary toxicity, genotoxicity, and carcinogenicity evaluation of molybdenum, lithium, and tungsten: A review

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ABSTRACT

Molybdenum, lithium, and tungsten are constituents of many products, and exposure to these elements potentially occurs at work. Therefore it is important to determine at what levels they are toxic, and thus we set out to review their pulmonary toxicity, genotoxicity, and carcinogenicity. After pulmonary exposure, molybdenum and tungsten are increased in multiple tissues; data on the distribution of lithium are limited. Excretion of all three elements is both via faeces and urine. Molybdenum trioxide exerted pulmonary toxicity in a 2-year inhalation study in rats and mice with a lowest-observed-adverse-effect concentration (LOAEC) of 6.6 mg Mo/m^3. Lithium chloride had a LOAEC of 1.9 mg Li/m^3 after subacute inhalation in rabbits. Tungsten oxide nanoparticles resulted in a no-observed-adverse-effect concentration (NOAEC) of 5 mg/m^3 after inhalation in hamsters. In another study, tungsten blue oxide had a LOAEC of 63 mg W/m^3 in rats. Concerning genotoxicity, for molybdenum, the in vivo genotoxicity after inhalation remains unknown; however, there was some evidence of carcinogenicity of molybdenum trioxide. The data on the genotoxicity of lithium are equivocal, and one carcinogenicity study was negative. Tungsten seems to have a genotoxic potential, but the data on carcinogenicity are equivocal. In conclusion, for all three elements, dose descriptors for inhalation toxicity were identified, and the potential for genotoxicity and carcinogenicity was assessed.

1. Introduction

Molybdenum, lithium, and tungsten are constituents of industrial products such as lubricating agents in spray form. Besides being used in industry, the transition metal molybdenum is an essential trace element, which binds to molybdopterin cofactors to serve a role in several enzymes (Paquet et al., 2016). The alkali metal lithium is used in the treatment of bipolar disorders (López-Muñoz et al., 2018) and is a main constituent of car-batteries. Tungsten (chemical symbol: W for wolfram) binds molybdopterin cofactors in bacteria and other organisms, but it has not yet been shown to be an essential trace element in humans. Tungsten also occurs naturally in groundwater in some places (Seiler, 2012). In addition to via lubricating processes, occupational exposure to tungsten occurs in metal industries (Klasson et al., 2016; Sahle et al., 1996), and via welding fumes (Graczyk et al., 2016). Soldiers are exposed to tungsten during combat due to its presence in some ammunition (Gold et al., 2007). Hard metal lung disease has been observed after exposure to hard metal. Hard metal has been described as a 'synthetic compound that combines tungsten carbide with cobalt as well as a number of other metals'. A unique characteristic of this hard metal lung disease has been reported to be giant cell interstitial pneumonia (Moriyama et al., 2007).

During manufacture and use, workers are potentially exposed to these elements via inhalation. Therefore, it is important to determine at what inhalation levels they are toxic. We set out to review the pulmonary toxicity, including toxicokinetics, of molybdenum, lithium, and tungsten. We also reviewed their genotoxicity following all exposure pathways and in vitro. The inclusion of all exposure pathways and in vitro studies is justified by the severity of the endpoint of cancer and because genotoxic effects are most often the result of processes inside the single cells, irrespective of the route of exposure.

2. Methods

Literature search strategy: We used combinations of the metal names and 'inhalation', 'pulmonary toxicity', 'genotoxicity', 'cancer', and

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'carcinogenicity' in the PubMed and Web of Science databases, as described in Supplementary materials Table S1. We also reviewed the reference list of retrieved articles to identify articles not found by the search strategies. In total, 122 articles were included in the current work.

3. Absorption, distribution, metabolism, and excretion (ADME)

3.1. Molybdenum

3.1.1. Normal levels of molybdenum in the body and levels after specific exposure

The mean normal level of Mo in blood of the studies cited in Fig. 1 is 14 μg/L, while that in serum was 20 μg/L. The normal human urine level of molybdenum is around 50 μg/L, while the 95th percentile is around 150 μg/L (Fig. 1).

Workers at a molybdenite roasting plant had increased molybdenum in plasma and urine. The plasma level ranged between 9 and 365 μg/L (mean level: 122 μg/L), while controls were in the range of 4–35 μg/L. Their urine concentration ranged between 120 and 11,000 μg/L (mean level: 1790 μg/L); normal urinary molybdenum ranged between 20 and 230 μg/L (mean level: 53 μg/L) (Walravens et al., 1979).

In rats and mice, molybdenum was increased after inhalation of molybdenum trioxide (MoO_3) (6.6, 20, or 66 mg Mo/m^3, 6 h/day, 5 days/week for 2 years). The data are depicted in Fig. 2 (Chan, 1998; NTP, 1997). The tissue distribution of ^{99}Mo was investigated in three dogs. After 8 days of recovery, 2% of the dose was detected in the lungs, while liver, kidney, muscle, and skeleton collectively contained 79 % of the dose. Molybdenum chloride was investigated in another 3 dogs, and 6% was found in the lungs, while 68 % was seen in the aforementioned organs. Lastly, in yet another 3 dogs, molybdenum trioxide exposure resulted in 46 % in the lungs and 39 % in the other organs (Cuddihy et al., 1969).

3.1.2. Excretion of molybdenum

Urine molybdenum gradually declined over 8 days in seven workers who accidentally inhaled ^{99}Mo (Alvarez et al., 1994). Injection studies indicate that the faecal excretion route plays a role too: after injection of ^{99}Mo in two patients, excretion occurred mainly through urine but also via faeces (Rosoff and Spencer, 1964). Urinary and faecal excretion were demonstrated after intravenous injection in pigs and cattle, with the urinary route being most pronounced in pigs and the faecal one most pronounced in cattle (Bell et al., 1964). Placental transfer was very low in one oral study in pigs (Shirley et al., 1954).

3.2. Lithium

3.2.1. Normal levels of lithium and levels after absorption

In Fig. 3, we present the normal levels of lithium in blood and urine as well as some organs. The mean serum level of four studies is 13 μg/L. Tissue levels vary between 0.002 mg/kg in the brain up to the milligram per kilo range in other tissues (Fig. 3). Concerning absorption of lithium after inhalation: 27 patients were ventilated by an apparatus that had a lithium-chloride-coated (LiCl) heat and moisture exchanger. Lithium was increased in serum, where it oscillated between 70 and 700 μg/L (Rosi et al., 1995).

3.2.2. Excretion of lithium

Urine is an excretion pathway at least at normal exposure levels, demonstrated by the presence of lithium in human urine (Fig. 3). Moreover, one study with intraperitoneal lithium injection demonstrated faecal excretion in rats, involving biliary excretion (urinary excretion was also seen) (Kersten and Barth, 1982), while non-biliary intestinal excretion was seen in another study with rats after intravenous lithium injection (Kersten et al., 1986).

3.3. Tungsten

3.3.1. Normal levels of tungsten in the body and levels in exposed animals

The mean normal blood level based on three investigations is 0.12 μg/L (Fig. 4). There is only limited information on tungsten in different organs in humans, and only skin is reported here (Fig. 4).

Rats were exposed to sodium tungstate ($Na_2{}^{188}WO_4$) at 256 mg W/m^3 for 90 min. Tissue levels were highest in the thyroid gland and in urine, but tungsten was also found in the kidneys, adrenal glands, spleen, femur, lymph nodes, and the brain. The organs continued to accumulate small amounts of tungsten throughout a 21-day follow-up period (Radcliffe et al., 2010). In the same experimental setup, the olfactory pathway was found to play a minimal role in the delivery of tungsten to the brain (Radcliffe et al., 2009). In another study, rats inhaled tungsten blue oxide[1] at ~63 and 514 mg W/m^3 for 6 h. Tungsten blood levels were ~800 and 10,900 μg/L blood in the low and high doses, respectively. Over the course of 144 h of recovery, these levels decreased to approximately ~5 and 100 μg/L, respectively (Rajendran et al., 2012).

3.3.2. Excretion of tungsten

The urinary pathway is demonstrated by the presence of this metal in urine in normal individuals (Fig. 3). Thirty-three workers were exposed to tungsten and cobalt when producing either hard-metal mechanical parts or diamond tools. In the workers' breath, tungsten ranged from undetectable to ~18.4 μg/L (the maximum cobalt level was 58.9 μg/L). Tungsten was not detected in the breath of controls, while cobalt was. Both metals were higher in urine after a work shift than before (Goldoni et al., 2004). In the aforementioned animal study with inhalation of tungsten blue oxide in rats, this metal dose-dependently increased in faeces, while the urine levels of tungsten were stated to be three orders of magnitude lower than in faeces (exact values were not reported) (Rajendran et al., 2012).

4. Pulmonary toxicity

4.1. Molybdenum

4.1.1. Data from studies with humans

Ott and co-workers studied 43 workers who were exposed to molybdenum trioxide at a metal plant. Thirty-three of the workers had respiratory symptoms. Lung X-rays showed no signs of toxicity, but workers who were symptomatic had elevated neutrophil and lymphocyte numbers in their bronchoalveolar lavage (BAL) fluid. This was seen both in comparison with workers who were asymptomatic and in comparison to controls. Workers exposed to molybdenum trioxide had a higher forced expired volume in the first second (FEV_1-%) and a forced vital capacity (FVC-%) than controls. There were no differences in lung function between symptomatic and asymptomatic workers (Ott et al., 2004).

4.1.2. Data from studies with animals

Molybdenum trioxide was dosed to rats and mice by inhalation for 14 days, 13 weeks, or 2 years. In the 14-day study, rats were exposed to 3, 10, 30, 100, or 300 mg MoO_3/m^3 (= 2, 6.6, 20, 66 or 200 mg Mo/m^3) for 6 h/day, 5 days/week. Compared with controls, the body weight was lower in the two highest dose-groups in males (and highest dose in females); but no other clinical signs were seen. In mice, the same dosage regimen resulted in lower body weight at the highest dose—as compared to controls, also in the absence of clinical findings. In the 13-week study, rats were exposed to molybdenum trioxide at 0.66, 2, 6.6, 20, or 66 mg Mo/m^3 (6.5 h/day, 5 days/week for 13 weeks). No differences were seen

[1] The compound used in that article consisted of 69% WO_3, 8.0% $W_{25}O_{73}$, and 23.0% $W_{20}O_{58}$.

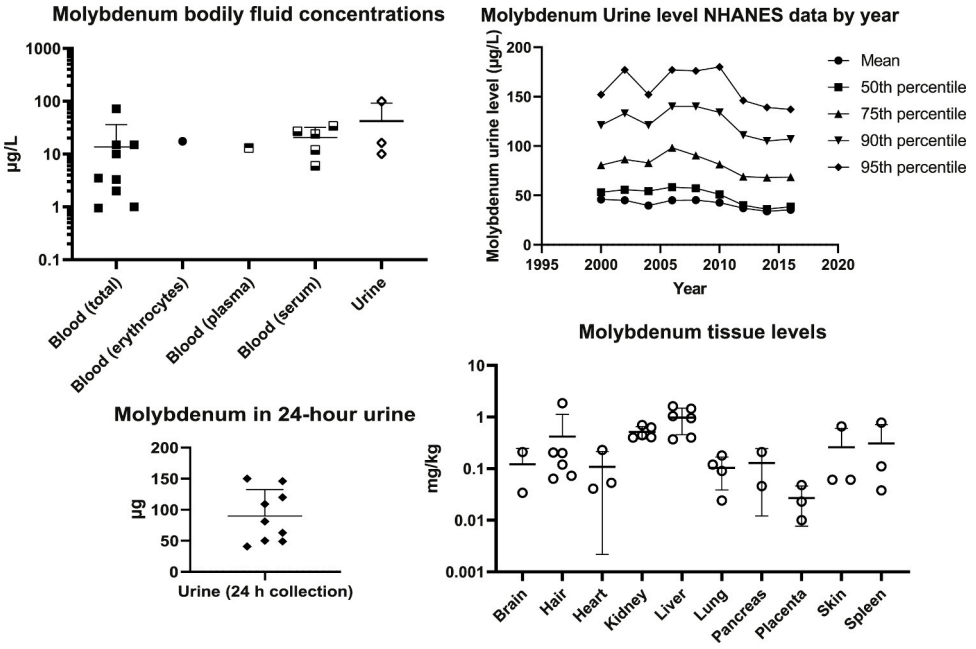

Fig. 1. Normal levels of molybdenum in human bodily fluids and organs. The graph is based on data collected by (Lyengar et al., 1978) supplemented with (Brune et al., 1966; Butt et al., 1964; Rentschler et al., 2018), and the NHANES data by (NHANES, 2019). Each data point represents a separate study. Data measured on dry tissue were converted to per fresh tissue by use of conversion factors given in Lyengar et al.

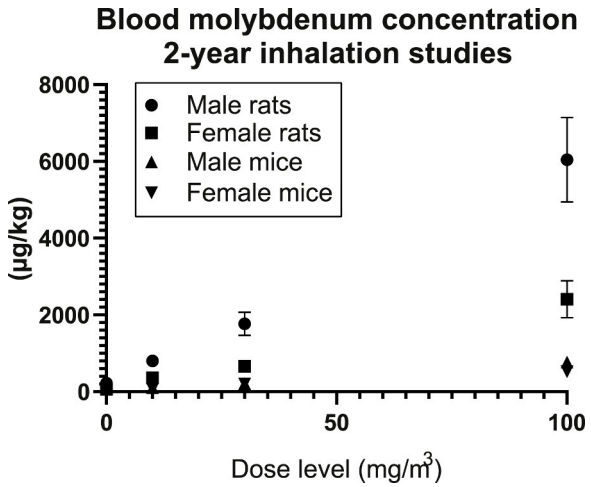

Fig. 2. Molybdenum blood concentration in a 2-year inhalation study in mice and rats. Data are from (NTP, 1997).

in body weight, organ weight, or clinical signs. Nor were clinical biochemistry, haematology, sperm counts, or sperm motility affected. Mice in the same experimental setup displayed no effects, except copper was increased in the liver of females at 20 and 66 mg Mo/m^3 and in males at 66 mg Mo/m^3. The 2-year study in rats (6.6, 20 and 66 mg Mo/m^3) is described in detail in the section on carcinogenicity, but we note here that chronic inflammation occurred at 20 and 66 mg Mo/m^3, providing a no-observed-adverse-effect concentration $(NOAEC)_{chronic\ inflammation}$ of 6.6 mg Mo/m^3 (equal to 10 mg MoO_3/m^3). Notably, in that study, various other lesions seen by histopathology were already seen at 6.6 mg MoO_3/m^3 in both rats and mice and could warrant a lowest-observed-adverse-effect concentration $(LOAEC)_{histopathological\ lesions}$ of 6.6 mg Mo/m^3 (Chan, 1998; NTP, 1997).

Molybdenum (IV) sulphide (MoS_2) nano- and microparticles were given to rats by single intratracheal instillation of 0.9 or 3 mg Mo/kg bw. Histopathology revealed inflammatory changes in the respiratory system, while biochemical and haematological endpoints were unaffected (Sobańska et al., 2020).

4.2. Lithium

4.2.1. Data from studies with animals

Rabbits were exposed to lithium chloride by inhalation of 0.6 and 1.9 mg Li/m^3, 6 h/day, 5 days/week for 4–8 weeks, and showed no inflammatory changes in the lung ($NOAEC_{inflammatory\ changes}$: 1.9 mg Li/$m^3$) (Johansson et al., 1988). Rats, mice, guinea pigs, and rabbits inhaled lithium hydride (LiH) at doses between 5 and 55 mg/m^3 (= 4.3 and 47 mg Li/m^3) for 4–7 h, or at 5 mg/m^3 for one week. All dose levels caused sneezing and coughing. Mortality was seen for 2 of 10 rats at 22 mg/m^3 and 4 of 10 rats at 36 mg/m^3. However, no effects on mortality were seen at three higher dose levels: 45, 49, and 55 mg/m^3. Post-exposure periods of up to 5 months did not indicate chronic effects. The authors of the study concluded that lithium hydride had irritating and corrosive effects, which could be ascribed to the alkalinity of the hydrolysis product. We have not set a dose descriptor (LOAEC) based only on sneeze and cough in this study (Hodge et al., 1956).

Particles of $LiFePO_4$, $Li_4Ti_5O_{12}$, or lithium cobalt oxide ($LiCoO_2$), with mass median particle geometric diameters between 4 and 8 µm, were given to mice by oropharyngeal aspiration of 0.5 or 2 mg (= 1 and 4 mg Li/kg bw for $LiFePO_4$, 1.6 and 6 mg Li/kg bw for $Li_4Ti_5O_{12}$, and 1.75 and 7 mg Li/kg bw for lithium cobalt oxide). In addition, lithium chloride was dosed at 6.9 mg Li/kg bw. Three days after exposure, inflammation in the form of elevated neutrophil numbers in bronchoalveolar (BAL) fluid was increased after the highest dose of lithium cobalt oxide but not after other particles. Two months after exposure, neutrophils were increased only after $LiFePO_4$. Subsequently, lithium chloride was investigated in a 2-month study and did not affect neutrophils (Sironval et al., 2018).

To simulate a fire in the containment building of a fusion reactor, lithium aerosols were generated by the burning of lithium metal. Rats inhaled aerosols at 500, 750, 1000, or 1500 mg/m^3, which consisted either of 1) lithium monoxide (LiO), lithium hydroxide, and lithium carbonate (Li_2CO_3) or 2) lithium hydroxide and lithium carbonate. Both aerosols resulted in histopathologic lesions in the nasal turbinates,

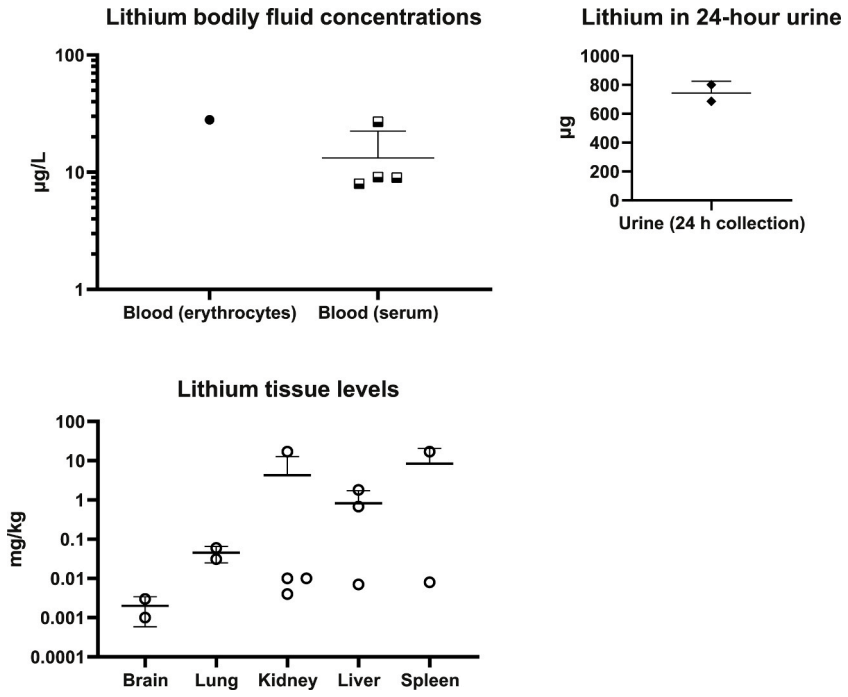

Fig. 3. Normal levels of lithium in human bodily fluids and organs. The graph is based on data collected by (Lyengar et al., 1978). Each data point represents a separate study. Data measured on dry tissue were converted to per fresh tissue by use of conversion factors from Lyengar et al. (1978).

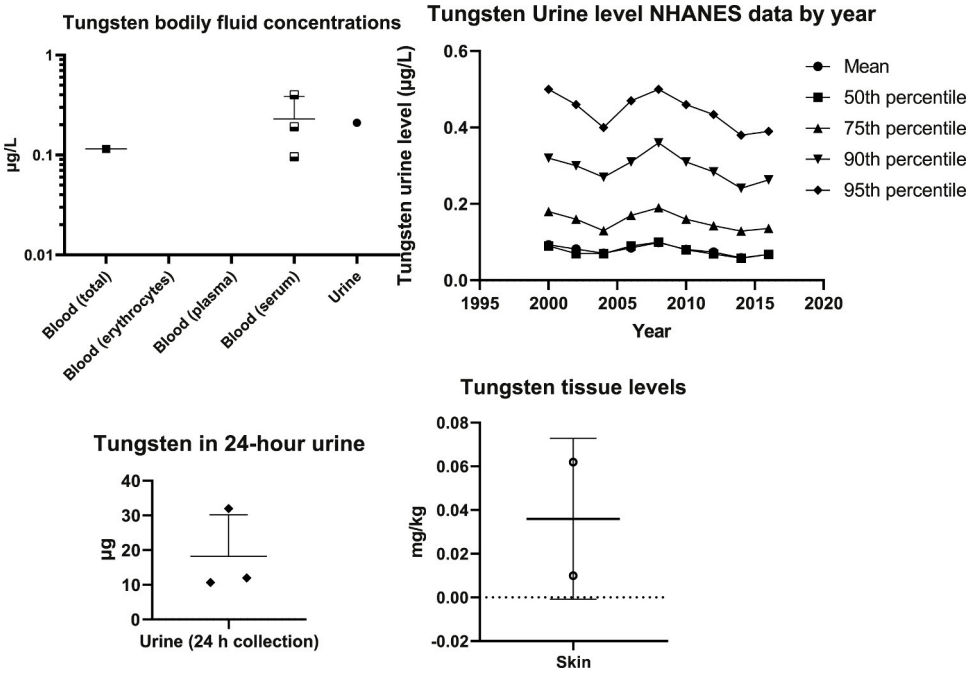

Fig. 4. Normal levels of tungsten in human bodily fluids and organs. The graph is based on data collected by (Lyengar et al., 1978) and the NHANES data by (NHANES, 2019). Each data point represents a separate study. Data measured on dry tissue were converted to per fresh tissue by use of conversion factors from Lyengar et al. (1978).

larynx, and lungs. The 4 -h lethal concentrations 50 % (LC_{50}) were based on the doses calculated to be more than 900 mg/m^3 (Rebar, 1986). The same research group tested a lithium combustion aerosol in rats at 620, 1400, 2300, or 2600 mg/m^3 (content: ~80 % lithium carbonate, ~20 % lithium hydroxide) for 4 h. After 14 days, the LC_{50} values were estimated to be 2000 mg/m^3 in females and 1700 in males. Clinical signs were anorexia, dehydration, respiratory difficulty, and perioral and perinasal encrustation. Histopathological effects were seen in the respiratory tract. In animals observed for a further 2 weeks, body weight, organ weights (initially depressed), and clinical signs began to normalise (Greenspan et al., 1986).

4.3. Tungsten

4.3.1. Tungsten pulmonary toxicity data from animal studies

Hamsters inhaled tungsten (IV) oxide (WO_3) nanoparticles at 5 or

10 mg/m^3, 4 h/day for 4 or 8 days. Affected endpoints included bronchoalveolar (BAL) fluid cellularity, total protein, lactate dehydrogenase and alkaline phosphatase activities, as well as the level of tumour necrosis factor-alpha. BAL neutrophils along with other markers such as lactate dehydrogenase (LDH) and total protein both in BAL fluid were increased only at 10 mg/m^3, while some markers, including alkaline phosphatase, were increased already at 5 mg/m^3 ($NOAEC_{BAL\ neutrophils}$: 5 mg/m^3) (Prajapati et al., 2017). Tungsten blue oxide[2] was given to rats at high dose levels ~63, 257 and 514 mg W/m^3, 6 h/day for 28 days. Recovery periods were 8 and 14 days. Neutrophils and monocytes were increased in blood at the highest dose level; eosinophils were increased at the two highest dose levels. Some effects were seen in clinical chemistry, in particular at the two highest doses. Lung weights were increased at all dose levels in males and at the two highest levels in females. Histopathological findings were seen at all dose levels in the form of alveolar foreign material, alveolar pigmented macrophages, and alveolar foamy macrophages ($LOAEC_{lung\ weight/histopathological\ findings}$: 63 mg W/m^3, the lowest dose level tested) (Rajendran et al., 2012).

Rats were exposed by intratracheal instillation to 20 mg/kg bw of either pure tungsten, nickel, or cobalt; or to combination-materials: 1) tungsten (92 %), nickel (5 %), and cobalt (3 %) (WNiCo) or 2) a powder consisting of WNiFe. Neutrophil numbers were increased in BAL fluid after the combination-materials but not after the individual metals (Roedel et al., 2012). Calcium tungstate ($CaWO_4$) particles were given to mice by intratracheal instillation of 8 mg W/kg bw. The total number of cells in BAL fluid was increased compared to control, and inflammation was observed in the bronchoalveolar space (Peão et al., 1993).

4.3.2. Hard metal disease: data from studies in humans and animals

Moriyama et al. described the distribution of inhaled hard metals and the accompanying inflammatory cells in lung tissue in 17 hard metal lung disease patients—shown to have giant cell interstitial pneumonia. Tungsten and cobalt were observed in fibrotic lesions in the lungs of all patients but not in five controls. Lymphocytes and macrophages surrounded the fibrotic lung lesions (Moriyama et al., 2007). Morfeld and colleagues studied a cohort of workers in the German hard metal industry to look at the relation between cobalt exposure in the presence and absence of tungsten and the risks of cause-specific and total mortality. The standardised mortality ratio (SMR) was elevated for non-malignant respiratory mortality and heart diseases. The risk of lung cancer was not elevated. There were no associations in relation to tungsten exposure (Morfeld et al., 2017). Lasfargues et al. investigated a cohort of workers who produced hard metals with the aim of assessing the lung cancer risk in relation to cobalt exposure. The overall SMR was 1.02, the SMR for lung cancer was 2.13, and the mortality was reported to be higher for workers placed in high exposure areas. However, there was no trend with the duration of employment (Lasfargues et al., 1994). In an epidemiological study, 23 patients with hard metal pneumoconiosis had various radiographic findings and signs of giant cell interstitial pneumonia (Chiarchiaro et al., 2018).

Notably, giant cell interstitial pneumonia is seen in some patients with no exposure to hard metals. Three of 455 people with transplanted lungs showed giant cell interstitial pneumonia, and none of the samples taken from the explanted lungs contained tungsten; only one had elevated cobalt (Khoor et al., 2016).

4.3.3. Tungsten carbide pulmonary toxicity in animal studies

Only studies with intratracheal instillation were identified, and they mostly showed no effect: Rats were exposed to tungsten carbide (WC) particles by intratracheal administration at a single dose of 9.3 mg W/kg bw. No effects were seen on BAL fluid neutrophils or macrophages, nor on BAL fluid LDH, total protein, or albumin (Huaux et al., 1995). Rats were instilled intratracheally with 50 μm particles of either tungsten carbide (10 mg/kg bw) or cobalt-tungsten carbide (WC-Co) (cobalt 6.3 %, tungsten 84 %, carbon 5.4 %; 167 mg/kg bw). Cobalt-tungsten carbide, but not tungsten carbide, increased neutrophils, macrophages, total protein, albumin and LDH in BAL fluid (Lasfargues et al., 1992). In a similar setup, doses were 9.3, 46.5, or 93 mg W/kg bw. Neutrophil numbers were increased at the two highest doses of tungsten carbide on day 1 but not day 28. The effect of cobalt-tungsten carbide persisted throughout day 28. LDH, albumin, and total protein in BAL fluid were slightly increased at the highest dose of tungsten carbide, and at all doses of cobalt-tungsten carbide at day 1 (and tungsten carbide also on day 28). The two particle types were also dosed at 10 mg/kg bw/month for 4 months, and no effects were observed on the above-mentioned parameters (Lasfargues et al., 1995). In mice, no effect was seen on neutrophil numbers in BAL fluid after intratracheal instillation of tungsten carbide at doses between 34.9 and 116.3 mg W/kg bw (Lardot et al., 1998).

5. Genotoxicity and carcinogenicity

5.1. Molybdenum

5.1.1. Genotoxicity

An overview table of the data is provided in supplemental materials Tables S2–4. Notably, we use both the terms ‘chromosomal aberrations’ and ‘clastogenicity’ in the genotoxicity sections. We consider clastogenicity to cover the term ‘structural chromosomal aberrations’ and ‘aneugenicity’ to cover ‘numerical chromosomal aberrations’, while we consider ‘chromosomal aberrations’ to cover both groups. However, in the text, we retain the wording of the original articles to precisely convey what the authors of the studies wrote.

5.1.2. In vitro—bacterial assays

Positive effects Molybdenum trioxide was mutagenic in *Salmonella typhimurium* TA98 and TA100 strains (Terpilowska and Siwicki, 2018), and molybdenum disulphide flakes were weakly positive in Ames Fluctuation test of reverse mutations in *Escherichia coli* TA100 (Appel et al., 2016). Ammonium molybdate was positive in *Escherichia coli trp-*, and ammonium molybdate and K_2MoO_4 were positive in the rec-assay in *Bacillus subtilis*, while molybdenum (V) chloride ($MoCl_5$) was negative (Nishioka, 1975). Finally, sodium molybdate was positive in the multi-endpoint Microscreen assay in *Escherichia coli* WP2s(λ) (Rossman et al., 1991).

Negative effects Molybdenum nano- and microparticles were negative in five bacterial strains and one cell transformation assay (Hasegawa et al., 2012). Molybdenum trioxide was not mutagenic in five strains of *Salmonella typhimurium* (NTP, 1997). Sodium molybdate (Na_2MoO_4) was negative in five *Salmonella typhimurium* strains (Burzlaff et al., 2017). Molybdenum trioxide, molybdenum sulphide and molybdic acid (H_2MoO_4) were negative in the *Bacillus subtilis* rec-assay, while ammonium molybdate ($(NH_4)_6Mo_7O_{24}$ was positive (Kanematsu et al., 1980). Ammonium molybdate was negative in *Salmonella typhimurium* and *Escherichia coli* fluctuation assays (Arlauskas et al., 1985). Sodium molybdate dihydrate had no effect in five *Salmonella typhimurium* strains (Burzlaff et al., 2017).

5.1.3. In vitro assays—mammalian cells

Positive effects Ammonium molybdate and, to a lesser extent, sodium molybdate caused increased micronuclei in binucleated lymphocytes. (Titenko-Holland et al., 1998).

Negative effects Molybdenum ions did not induce double-strand breaks in comet assay in human T-lymphocytes (Caicedo et al., 2008). Molybdenum trioxide had no effects on chromosome aberrations or sister chromatid exchange in Chinese hamster ovary cells (NTP, 1997), nor was it positive in the micronucleus and comet assays in both HepG2 and BALB/3T3 cells (Terpilowska and Siwicki, 2018). Molybdenum

[2] The compound used in the reported article consisted of 69% WO_3, 8.0% $W_{25}O_{73}$, and 23.0% $W_{20}O_{58}$.

trioxide concentration-relatedly increased micronuclei in Syrian hamster embryo cells (Gibson et al., 1997). Sodium molybdate (Na_2MoO_4) was not mutagenic in L5178Y mouse lymphoma cells and did not increase micronuclei numbers in human peripheral blood lymphocytes (OECD guideline study) (Burzlaff et al., 2017). Sodium molybdate dihydrate was not mutagenic or clastogenic in mouse lymphoma assay in L5178Y cells (Burzlaff et al., 2017). Finally, Co-Cr-Mo-alloy had no effect on the comet and cytokinesis-block micronucleus assays in human lymphocytes (Gajski et al., 2014).

5.1.4. In vivo genotoxicity assays

Positive effects Molybdenum nanoparticles were dosed orally to mice at 500 mg/kg bw, and DNA damage was seen by Comet assay in the liver, brain, and bone marrow cells (Mohamed et al., 2020). Sodium molybdate was dosed to mice by twice intraperitoneal injection at total doses of 200 or 400 mg/kg (bw). Twenty-four hours later, micronuclei induction was increased in bone marrow at both doses, and the compound was also positive in the dominant lethal assay. (Titenko-Holland et al., 1998). $MoCl_3$ was positive in *Drosophila melanogaster* in the wing spot test (Iyehara Ogawa et al., 1994).

Negative effects Molybdenum (IV) sulphide nano- and microparticles had no effect in the comet assay when given to rats by single intratracheal instillation of 1.5 or 5 mg/kg bw (Sobańska et al., 2020).

5.1.5. Human studies

DNA damage was measured in lymphocytes from people exposed in a metalloid mining area in which molybdenum blood levels are known to be increased along with other elements (e.g., arsenic, chromium, lead, manganese, and zinc). Increased DNA damage was observed in the comet, micronucleus, and chromosomal aberration assays. Environmentally exposed people generally had higher DNA damage than in controls (Coelho et al., 2013). Buccal cells from people who had a Co-Cr-Mo dental casting alloy were found to have increased DNA damage in the comet assay compared to a control group (Baričević et al., 2012). In both studies, it is unknown if the molybdenum drives the effect.

5.1.6. Conclusion on the genotoxic potential of molybdenum

Molybdenum seems to be negative for genotoxicity in vitro—as numerous studies using various molybdenum compounds were negative, and only a few studies were positive. However, three of four in vivo studies are positive, using different molybdenum compounds and in different assays: *Drosophila melanogaster* wing spot test, micronucleus assay, dominant lethal assay, and the comet assay. The only negative study was with the comet assay. The few available in vivo studies show that genotoxicity cannot be excluded after oral and intraperitoneal exposure. The negative study used intratracheal instillation as the exposure route. The positive studies did not apply an exposure route relevant for inhalation; therefore, the in vivo genotoxicity after inhalation remains unknown and more studies are needed to make a firm conclusion.

5.1.7. Carcinogenicity of molybdenum

Molybdenum trioxide was investigated in rats and mice at inhalation levels of 6.6, 20, or 66 mg Mo/m^3 (6 h/day 5 days/week for 2 years). In male rats, a range of incidences of respiratory system neoplasms and non-neoplastic lesions in rats were increased already at the lowest dose. These were squamous metaplasia of the epithelium lining the base of the epiglottis in the larynx, while there were no increases in alveolar/bronchiolar adenoma or carcinoma. In female rats, there were increased incidences already at the lowest dose in squamous metaplasia of the epithelium lining the base of the epiglottis and nose. Also, there were no increases in alveolar/bronchiolar adenoma or carcinoma in female rats.

In mice, some incidences of selected respiratory system neoplasms and non-neoplastic lesions were increased at 6.6 mg Mo/m^3. In male mice, squamous metaplasia in the epiglottis, lung infiltration cellular, histiocyte, and lung alveolar epithelium metaplasia were increased at the lowest dose level. The incidence of lung carcinoma was increased at all doses of male mice, and the incidence of carcinoma/adenoma combined was statistically significantly increased at the two lowest doses in male mice (control: 11/50), low dose (27/50), mid-dose (21/49) high dose 18/50. In female mice, squamous metaplasia in the epiglottis and Alveolar/bronchiolar epithelium metaplasia were already increased at the lowest dose. The incidence of adenoma was increased at the two highest doses, while the combined incidence of adenoma-carcinoma was increased only at the highest dose (control (3/50), low dose (5/50), mid-dose (8/49) and high dose (15/49)).

In conclusion, there was no evidence of carcinogenicity in rats—based on no increase in alveolar/bronchiolar adenoma or carcinoma. By contrast, there was evidence of carcinogenicity in both male and female mice. Based on the data, a NOAEC could be set on inflammation in rats of 6.6 mg Mo/m^3, but notably, carcinogenicity may occur already at this dose level (6.6 mg Mo/m^3) (based on lung carcinomas in male mice) (Chan, 1998; NTP, 1997).

Whether a potential carcinogenic effect occurs through a threshold or non-threshold mechanism is unknown. Among the in vivo genotoxicity studies, only one study (negative result) used an exposure route (intratracheal instillation) that was relevant for inhalation exposure. Therefore, more studies are required to assess the mechanism behind the carcinogenic effects in mice after inhalation of molybdenum.

Concerning other exposure pathways: Molybdenum trioxide was given to mice by 19 intraperitoneal injections (total doses: 627, 1805, 3135 mg Mo/kg bw). The animals were killed 30 weeks after the first injection. The number of lung tumours per mouse (multiplicity) was increased at the highest dose group 3135 mg Mo/kg bw. Vehicle treated (control) mice had 0.42 tumours per mouse, and in the high dose groups, this number was 1.13). However, the incidence (number of mice with tumours) was no different from control though (Stoner et al., 1976). Molybdenum trioxide was evaluated by International Agency for Research on Cancer (IARC) in 2018, and as no data on cancer in humans were identified, molybdenum trioxide was evaluated as Group 2B (possibly carcinogenic to humans) (IARC, 2018).

After subcutaneous injection of 30 mg molybdenum orange (lead chromate, sulphate, and molybdate), tumours were observed in 36 of 40 rats with an average latency time of 32 weeks (experiment length: 117 weeks). The histology type was rhabdomyosarcomas and fibrosarcomas. In controls, there were no animals with tumours after 127 weeks (Maltoni, 1976).

5.1.8. Conclusion on the carcinogenic potential of molybdenum

There is evidence in mice for a carcinogenic effect after inhalation of molybdenum trioxide. While there was no evidence for this effect in rats. Molybdenum trioxide was negative after intraperitoneal injection. This compound was evaluated IARC 2B, *possibly carcinogenic to humans*, by IARC in 2018. One combination of lead chromate, sulphate and molybdate was carcinogenic after subcutaneous injection. Overall, the data point towards a carcinogenic potential of this element.

5.2. Lithium

5.2.1. Genotoxicity

A tabulated overview of the data is provided in supplemental materials Tables S5–7.

5.2.2. Mutagenicity in bacterial cells

Negative effects Lithium hypochlorite (LiOCl) was negative in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 (Weiner et al., 1990). Lithium chloride was negative in the *Bacillus subtilis* rec-assay in two studies (Kanematsu et al., 1980; Nishioka, 1975), and trilithium citrate was negative in *Salmonella Typhimurium* and *Escherichia coli*/ mammalian-liver homogenate test (King et al., 1979).

5.2.3. In vitro data—mammalian cells

Positive effects Lithium chloride increased γH2AX, a marker of DNA double-strand breaks, in SH-SY5Y human neuroblastoma cells in a concentration-related manner (Stampone et al., 2020). Lithium carbonate- and lithium chloride-induced micronuclei but did not affect DNA strand breaks or chromosome breaks in CHO cells; both compounds showed an effect on mitosis (Pastor et al., 2009). Lithium cobalt oxide and $Li_4Ti_5O_{12}$ particles were tested in the cytokinesis-block micronucleus and the comet assay in rat lung epithelial cells. Lithium cobalt oxide-induced micronuclei and oxidative DNA strand breaks, while $Li_4Ti_5O_{12}$ did not (only one concentration tested). Notably, the effect of the former might be driven by Co (Sironval et al., 2020). Lymphocytes from a healthy donor were treated in vitro with lithium chloride and showed an increased incidence of chromosomal anomalies and a significant increase in satellite associations (De La Torre et al., 1976). When testing for gene conversion and reverse mutation (*trp 5* and *ilv1*) in *Saccharomyces cerevisiae,* lithium sulphate (Li_2SO_4) was weakly positive (Singh, 1983).

Negative effects Lithium hypochlorite was not mutagenic in the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) mutation assay in Chinese hamster ovary cells. Nor was it genotoxic in the unscheduled DNA synthesis assay in primary hepatocytes. The highest tested concentration produced chromosome aberrations at some concentrations in Chinese hamster ovary cells (Weiner et al., 1990). Lithium carbonate showed only weak genotoxicity in the assay of 6-thioguanine resistant mutants in mammalian cells (Slameňová et al., 1986). No effect was seen of lithium metaborate dihydrate in micronuclei or chromosomal aberration assays in human lymphocytes (Çelikezen et al., 2016). Lithium titanate (Li_2TiO_3) nanoparticles did not induce genotoxicity in human peripheral lymphocytes, measured as sister chromatid exchanges, micronuclei induction, and chromosome aberrations (Akbaba et al., 2016). Likewise, lithium titanate nanoparticles had no effect in the micronucleus test or on 8-oxo-2-deoxyguanosine levels in hepatocytes (Turkez et al., 2016).

5.2.4. In vivo genotoxicity assays

Positive findings Trilithium citrate increased micronuclei in mouse bone marrow after two intraperitoneal injections of 82 mg Li/kg bw, but not after two times of 41 or 20.5 mg Li/kg bw. Moreover, this compound was negative for sex-linked recessive mutants in *Drosophila melanogaster* (King et al., 1979). Lithium 0.05 % in drinking water (25 mg Li/kg bw/day) had a weak effect on unscheduled DNA synthesis in rat lymphocytes after 3, 6 and 12 months of exposure (Šrám et al., 1990). Lithium carbonate, lithium chloride and lithium acetate were tested in mice bone marrow cells after exposure for 72 h by oral gavage of lithium carbonate (0.2, 2.3 and 22.8 mg Li/kg bw) or lithium chloride (0.03, 0.3 or 3.3 mg Li/kg bw); or lithium acetate (0.005, 0.05 or 0.5 mg Li/kg bw). All three compounds caused chromosomal aberrations but did not affect sister chromatic exchanges in mice bone marrow cells (Sobti et al., 1989).

Negative findings Lithium hypochlorite had no effect on chromosome aberrations after intraperitoneal injection (5.9, 29.6, 59 mg Li/kg bw[3] in females and 11.8, 59, 118 mg Li/kg bw in males) (Weiner et al., 1990). Likewise, no chromosomal aberrations were observed in bone marrow cells from rats intraperitoneally injected with lithium (form not further specified, dose: 344 mg Li/kg bw per day for three days) (Bille et al., 1975).

5.2.5. Human studies

Eighteen patients who received lithium carbonate in combination with benzodiazepines and antipsychotic pharmaceuticals were tested for chromosomal aberrations and sister chromatid exchanges in peripheral blood. Both endpoints were increased as compared to controls. However, it is unknown if the effect is caused by any of the two non-lithium substances (Bigatti et al., 1998). Lymphocytes were isolated from 10 psychiatric patients who were taking Lithium carbonicum. The lymphocytes showed elevated chromosomal abnormalities as compared to cells from three control persons (De La Torre et al., 1976). Three psychiatric patients under treatment with lithium had increased breaks and hypo-diploid cells in leukocytes compared to a control group (11 persons) (Friedrich and Nielsen, 1969).

In response to the limited sample size in the former study, Jarvik et al. investigated 16 manic-depressive patients who were under treatment with lithium carbonate and found no statistical difference from controls concerning chromosome breaks (Jarvik, 1971). The frequency of chromosomal aberrations was unaffected in 13 patients who were on lithium therapy (Matsushima et al., 1986). No increase in chromosomal aberrations was seen in peripheral blood lymphocytes of 77 people taking lithium as a pharmaceutical compared to controls (Banduhn et al., 2008).

5.2.6. Conclusion on the genotoxic potential of lithium

The data on lithium in vitro are equivocal; there are several negative studies; however, there are also positive studies indicating a clastogenic effect. In vivo studies in animals show a similar number of negative and positive results. The studies showing a positive effect measure damage on chromosomes. Therefore, a clastogenic effect cannot be excluded after intraperitoneal and oral exposure. Some human studies show an association between lithium substances exposure and genotoxicity; however, it remains unknown if the effect was due to lithium substances, and better-designed human studies are required to make firm conclusions.

5.2.7. Carcinogenicity studies

A nationwide case-control study in Denmark found no association between long-term use of lithium as an anti-depressive drug (>5 years) and 36,248 cases of colorectal adenocarcinoma in the years 2000–2012 (Pottegård et al., 2016). Some studies point to decreased cancer incidence in patients with bipolar disorder who were treated with lithium (Cohen et al., 1998; Huang et al., 2016; Martinsson et al., 2016).

5.3. Tungsten

5.3.1. Mutagenicity in bacterial cells

Positive effects An overview table of the data is provided in supplemental materials Tables S8 and 9. Tungsten trioxide (WO_3) nanoplates were incubated with naked DNA plasmid. The plasmids were subsequently transfected into *Escherichia coli* to determine the mutation frequency. The nanoplates induced single and double-strand breaks and increased the mutation frequency (Thongkumkoon et al., 2014). Tungsten trioxide nanoparticles were tested along with other metals in five *Salmonella Typhimurium* strains. The nanosized tungsten trioxide particles, but not the micro-sized ones, induced weak mutagenicity (Hasegawa et al., 2012). Sodium tungstate showed effects in the bioluminescence mutation test in *Photobacterium fischeri* (Ulitzur and Barak, 1988).

Negative effect Tungsten disulphide (WS2) was not mutagenic in the *Salmonella typhimurium* TA100 strain (Appel et al., 2016).

5.3.2. In vitro studies—mammalian cells

Positive effects When measuring gene conversion and reverse mutation (*trp 5* and *ilv1*) in *Saccharomyces cerevisiae*, sodium tungstate showed a weak positive effect, while sodium molybdate did not (Singh, 1983). Pure small tungsten particles induced micronuclei in HepG2 cells; this was not seen with large tungsten particles. (Kühnel et al., 2012). Tungsten trioxide nano- and microparticles (54 nm and 3.9 μm) were tested in human lung carcinoma (A549) cells. WO_3 nanoparticles induced DNA strand breaks and micronuclei formation, whereas tungsten trioxide microparticles had no effect (Chinde et al., 2018). Tungsten

[3] This is likely per kg bw, but not stated in the article

and two combination materials were tested in a human osteoblast-like cell line. The compounds consisted of tungsten-nickel-cobalt or tungsten-nickel-iron. Tungsten alone, as well as the combination-materials induced micronuclei at all tested concentrations (Miller, 2001). Sodium tungstate ($Na2WO_4$) increased the recombination frequency in *Saccharomyces cerevisiae* (Sora et al., 1986). Tungsten was tested in a co-culture model involving a bone marrow stromal cell line and a primary bone marrow culture. Genotoxicity was observed by comet assay in a concentration-related manner, and increased protein level of γH2AX was seen too (Guilbert et al., 2011).

Negative effects Sodium tungstate, tested in Syrian hamster cells and human blood cultures, had no effect on sister chromatid exchange or chromosome aberration (Larramendy et al., 1981).

5.3.3. *In vivo studies*

Mice were exposed to tungsten for 8 weeks via drinking water (3.75 and 50 mg W/kg bw/day). The comet assay observed a dose-dependent effect in bone marrow cells. (Guilbert et al., 2011). Tungsten was dosed for 16 weeks via the drinking water (2.2, 30 or 150 mg W/kg bw/day), and DNA strand breaks were measured in bone marrow cells of male mice after 1, 4, 8, 12, and 16 weeks. After 4 weeks, there was an increase at all three doses; however at the other sampling times there was no increase at the highest dose (Kelly et al., 2013). Genotoxicity of tungsten trioxide nano- and microparticles was studied in an oral gavage animal study, in which rats were exposed to single doses of 80, 400, or 800 mg W/kg bw. In the micronucleus test, comet assay, and chromosomal aberration test, nanoparticles had an effect at the highest dose; the microsized particles had no effect (Chinde et al., 2017).

5.3.4. *Tungsten in combination with carbon and cobalt*

An alloy consisting of 91% tungsten (6% nickel, 3% cobalt) increased DNA strand breaks in the comet assay in human HSkMc primary muscle cells (Harris et al., 2015). Cobalt with tungsten carbide induced DNA damage in the comet assay in human lymphocytes (De Boeck, 1998). Tungsten carbide in combination with cobalt was tested in human lymphocytes with the comet assay and micronucleus test. There was no effect in the comet assay; however, there was an induction of micronuclei (De Boeck et al., 2003). Cobalt with tungsten carbide nanoparticles was tested in L5178Y mouse lymphoma cells and primary human lymphocytes. Some effects were observed in either type of cell in the micronucleus assay, chromosome aberration assay and the comet assay with and without formamidopyrimidine DNA glycosylase enzymes. (Moche et al., 2015). WC-Co nanoparticles 60 nm were tested in human liver (Hep3B) and kidney (Caki-1) cells. Genotoxicity was measured by γ-H2AX staining, and DNA double-strand breaks were seen in both cell lines (Paget et al., 2015). Cobalt-tungsten carbide and pure tungsten-carbide particles were tested for genotoxicity in the comet and micronucleus assays in isolated human leukocytes. Cobalt-tungsten carbide and cobalt alone both had an effect; tungsten alone had no effect in the comet assay. Both Cobalt-tungsten carbide and tungsten carbide induced micronuclei (Van Goethem et al., 1997). Cobalt-tungsten carbide particles, cobalt particles and tungsten-carbide particles were tested for genotoxicity in human peripheral lymphocytes. WC alone did not induce single strand DNA breaks, whereas cobalt particles alone did and so did cobalt-tungsten carbide (Anard, 1997). Cobalt-tungsten carbide nanoparticles were found to be genotoxic in primary cultures of human lymphocytes and in the L5178Y mouse lymphoma cell line using the mouse lymphoma assay, micronucleus test and the comet assay (Moche et al., 2014).

Cobalt-tungsten carbide particles were tested in rat lung epithelial cells, with only one concentration (50 μg/mL). There was a clear increase in micronuclei and DNA strand breaks with the enzyme hOGG1. However, there was no effect without the enzyme (Sironval et al., 2020). In the same study, induction of micronuclei in pneumocytes in female rats was also investigated by oropharyngeal aspiration, and cobalt-tungsten carbide particles induced a clear effect at the only dose tested; 2 mg/kg (Sironval et al., 2020). Cobalt-tungsten carbide dust was investigated in different types of lung cells with comet assay and micronucleus assay after intratracheal instillation. There was an effect in the induction of DNA strand breaks in pneumocytes after 12 h of exposure and in BAL cells after 12, 48, and 72 h, but no effect in peripheral blood mononuclear cells. Micronuclei formation was increased after 17 mg/kg bw exposure after 72 h, while no effect was observed at 1.8, 5.5, or 50 mg/kg bw (De Boeck, 2003).

5.3.5. *Conclusion on the genotoxic potential of tungsten*

There is evidence for in vitro genotoxicity of tungsten. There is also evidence of in vivo genotoxicity expressed as DNA strand breaks and clastogenic effects after the oral route. Tungsten in combination with cobalt shows in vivo effects after inhalation via relevant exposure routes.

5.3.6. *Carcinogenicity*

Rats were given tungsten tungstate in the drinking water at 5 ppm until natural death (=~0.25 mg *tungsten tungstate*/kg bw/day[4]). Equal doses of aluminium, barium, and beryllium were tested in parallel. Tungsten causes a slight decrease in longevity; however, the incidence of tumours was not increased. By contrast, the number of rats with tumours was increased for aluminium in male rats (Schroeder and Mitchener, 1975). Rats were implanted intramuscularly with tungsten alloy pellets (cylinders of 1 × 2 mm) at either 4 or 20 pellets/rat. Positive control animals had 20 pellets of nickel implanted. Within 4–5 months, the high dose tungsten dosed rats developed tumours surrounding the pellets in all animals. Tumours were also observed at the lower dose and in the positive controls, but this was a lower rate—but still all animals had tumours. Negative controls implanted with 20 pellets of tantalum did not develop tumours. The tumours were described as high-grade pleomorphic rhabdomyosarcomas. Metastases were observed in the lung of animals with tungsten (Kalinich et al., 2005). Bolt and colleagues showed that in a mouse breast cancer model, tungsten enhanced metastasis (Bolt et al., 2015). Rats had pellets of tungsten/nickel/cobalt, tungsten/nickel/iron, or pure tungsten, implanted into the muscle. Sarcomas were found around tungsten/nickel/cobalt pellets, whereas there was no effect after pure tungsten nor after tungsten/nickel/iron (Schuster et al., 2012).

Cobalt with tungsten carbide has been evaluated group 2A by IARC—probably carcinogenic to humans (IARC, 2006). A childhood cancer cluster in Fallon, Nevada, USA, discovered in the year 2000 was suggested to be associated with increased tungsten (Sheppard et al., 2007; Steinberg et al., 2007), but other causes may also have contributed (Daughton, 2005).

5.3.7. *Conclusion on the carcinogenic potential of tungsten*

One study indicated a carcinogenic potential of tungsten when rats were implanted intramuscularly with tungsten alloy pellets. However, rats exposed to tungsten via drinking water for their entire life did not develop tumours. A carcinogenic potential based on these two studies cannot be excluded.

6. Hazard characterisation

Key inhalation studies are summarised in Table 1.

6.1. *Molybdenum*

Molybdenum trioxide exposed workers had increased neutrophils and lymphocytes in BAL fluid and effects on lung function compared

[4] Using an European Food Safety Authority default value for converting test substance concentrations in drinking water (mg/L), into daily dose (mg/kg bw/day)(EFSA, 2012).

Table 1
Suggested dose descriptors from inhalation studies in rodents. Genotoxicity was not included in this table because it is not an endpoint but a mechanism for cancer and reproductive effects. *Concerning the carcinogenicity studies with molybdenum trioxide, we decided to set a LOAEC value, although we have not evaluated whether a potential carcinogenic mechanism of this substance is with a threshold.

Element and endpoint	NOAEC	LOAEC
Molybdenum		
Bodyweight lower than in controls	Molybdenum trioxide (MoO_3) in male rats (60 h) $NOAEC_{lower\ body\ weight}$ of 20 mg Mo/m^3 (Chan, 1998; NTP, 1997)	Molybdenum trioxide (MoO_3) in male rats (60 h) $LOAEC_{lower\ bodyweight}$ of 66 mg Mo/m^3 (Chan, 1998; NTP, 1997)
Chronic inflammation	Molybdenum trioxide (MoO_3) in rats (2-year study = 3120 h) $NOAEC_{chronic\ inflammation}$ of 6.6 mg Mo/m^3 (Chan, 1998; NTP, 1997)	Molybdenum trioxide (MoO_3) in rats (3120 h) $LOAEC_{chronic\ inflammation}$ of 20 mg Mo/m^3 (Chan, 1998; NTP, 1997)
Histopathologic lesions	No lower concentrations	Molybdenum trioxide (MoO_3) in male mice (3120 h) $LOAEC_{histopathological\ lesions}$ of 6.6 mg Mo/m^3 (Chan, 1998; NTP, 1997).
Lung carcinoma	No lower concentrations	Molybdenum trioxide (MoO_3) in male mice (3120 h) *$LOAEC_{Lung}$ carcinoma of 6.6 mg Mo/m^3 (Chan, 1998; NTP, 1997)
Lithium		
Inflammatory changes in lung	Lithium chloride (LiCl) in rabbits (120 to 240 h): $NOAEC_{inflammatory\ changes\ in\ lung}$: 1.9 mg Li/$m^3$ (Johansson et al., 1988).	No higher concentrations
Tungsten		
Increased BAL neutrophils	Tungsten (IV) oxide (WO_3) nanoparticles in hamsters (16 or 32 h) $NOAEC_{BAL\ neutrophils}$: 5 mg/m^3 (Prajapati et al., 2017)	Tungsten (IV) oxide (WO_3) nanoparticles in hamsters (16 or 32 h) $LOAEC_{BAL\ neutrophils}$: 10 mg/m^3 (Prajapati et al., 2017)
Increased blood neutrophils	Tungsten blue oxide in rats (168 h) $NOAEC_{blood\ neutrophils}$: 257 mg W/$m^3$ (Rajendran et al., 2012)	Tungsten blue oxide in rats (168 h) $LOAEC_{blood\ neutrophils}$: 514 mg W/$m^3$ (Rajendran et al., 2012).
Increased lung weight and histopathological findings in lung (alveolar foreign material, alveolar pigmented macrophages, and alveolar foamy macrophages)	No lower doses	Tungsten blue oxide in rats (168 h) $LOAEC_{lung\ weight/histopathological\ findings}$: 63 mg W/$m^3$, the lowest dose level tested (Rajendran et al., 2012).

with controls. However no doses were given in the article (Ott et al., 2004). In animal studies of molybdenum trioxide in 2- and 13-week studies, 66 mg Mo/m^3 was a NOAEC, except for male rats in the 2-week study in which this value was a LOAEC based on decreased bw (male rats NOAEC: 20 mg Mo/m^3). In the 2-year study (described in more detail in the section of carcinogenicity), however, chronic inflammation in rats occurred at 20 and 66 mg Mo/m^3, resulting in a $NOAEC_{chronic\ inflammation}$: 6.6 mg Mo/$m^3$. Notably, in that study, other histopathology lesions were already seen at 6.6 mg Mo/m^3 in both rats and mice and could warrant a $LOAEC_{histopathological\ lesions}$ of 6.6 mg Mo/m^3 (Chan, 1998; NTP, 1997). Genotoxicity cannot be excluded after Molybdenum exposure, and there was some evidence of carcinogenicity of molybdenum trioxide, starting at the lowest tested concentration, 6.6 mg Mo/m^3 (Chan, 1998; NTP, 1997).

6.2. Lithium

Lithium chloride by inhalation in rabbits had a NOAEC of 1.9 mg Li/m^3 (highest tested level) with a lack of inflammatory changes in the lung. (Johansson et al., 1988). The data on genotoxicity of lithium indicated that a genotoxic effect cannot be excluded, while one carcinogenicity study was negative.

6.3. Tungsten

Concerning tungsten, in hamsters that inhaled tungsten (IV) oxide nanoparticles gave a $NOAEC_{BAL\ neutrophils}$: 5 mg/m^3 (Prajapati et al., 2017). Moreover, inhaled Tungsten blue oxide at high doses to rats gave a ($LOAEC_{lung\ weight/histopathological\ findings}$: 63 mg W/$m^3$, the lowest dose level tested) (Rajendran et al., 2012). One special case of the potential involvement of tungsten in toxicity is hard metal disease. There is some evidence of toxicity after exposure in the hard metal industry; although, one study points towards tungsten not being involved in this effect. Tungsten seems to have a genotoxic potential, but the data on carcinogenicity is equivocal.

7. Summary

Molybdenum, lithium, and tungsten were reviewed, focusing on levels at which they become pulmonary toxic and for data on genotoxicity and carcinogenicity. However, the knowledge on the pulmonary toxicity of these three elements is limited. Molybdenum trioxide exerted effects in a 2-year inhalation study in rats and mice at 6.6 mg Mo/m^3. In mice, there was some evidence for a carcinogenic effect. The pulmonary toxicity data of lithium are limited. In one study, there was no effect of inhalation of lithium chloride at 1.9 mg Li/m^3, Lithium containing smoke had effects. Increased urinary levels of tungsten in the general population have been shown to be associated with increased risk for cardiovascular disease and stroke. Inhalation of tungsten oxide nanoparticles in hamsters provided a NOAEC of 5 mg/m^3, while tungsten blue oxide in rats at the lowest tested concentration, 63 mg W/m^3, increased the lung weight and exhibited histopathological changes in the lung. One aspect of tungsten is its presence in hard metal production—yet the evidence of tungsten toxicity in hard metal production seems weak. Concerning genotoxicity, for molybdenum, the in vivo genotoxicity after inhalation remains unknown; however, there was some evidence of carcinogenicity of molybdenum trioxide. The data on the genotoxicity of lithium are equivocal, while one carcinogenicity study was negative. Tungsten seems to have a genotoxic potential, but the data on carcinogenicity is equivocal.

CRediT authorship contribution statement

Niels Hadrup: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Jorid B. Sørli:** Conceptualization, Writing – review & editing. **Anoop K. Sharma:** Conceptualization, Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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