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Scientific Basis for Swedish Occupational Standards XXX

Swedish Criteria Group for Occupational Standards
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Translation:
Frances Van Sant
(except for the consensus report on Grain dust which was originally written in English)

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Preface

These documents have been produced by the Swedish Criteria Group for Occupational Standards, the members of which are presented on the next page. The Criteria Group is responsible for assessing the available data that might be used as a scientific basis for the occupational exposure limits set by the Swedish Work Environment Authority. It is not the mandate of the Criteria Group to propose exposure limits, but to provide the best possible assessments of dose-effect and dose-response relationships and to determine the critical effect of occupational exposure.

The work of the Criteria Group is documented in consensus reports, which are brief critical summaries of scientific studies on chemically defined substances or complex mixtures. The consensus reports are often based on more comprehensive criteria documents (see below), and usually concentrate on studies judged to be of particular relevance to determining occupational exposure limits. More comprehensive critical reviews of the scientific literature are available in other documents.

Literature searches are made in various databases, including Arbline, Chemical abstracts, Cheminfo, Medline, Nioshtic, RTECS and Toxline. Information is also drawn from existing criteria documents, such as those from the Nordic Expert Group (NEG), WHO, EU, NIOSH in the U.S., and DECOS in the Netherlands. In some cases the Criteria Group produces its own criteria document with a comprehensive review of the literature on a particular substance.

As a rule, the consensus reports make reference only to studies published in scientific journals with a peer review system. This rule may be set aside in exceptional cases, provided the original data is available and fully reported. Exceptions may also be made for chemical-physical data and information on occurrence and exposure levels, and for information from handbooks or documents such as reports from NIOSH and the Environmental Protection Agency (EPA) in the U.S.

A draft of the consensus report is written in the secretariat of the Criteria Group or by scientists appointed by the secretariat (the authors of the drafts are listed in the Table of Contents). After the draft has been reviewed at the Criteria Group meetings and accepted by the group, the consensus report is published in Swedish and English as the Criteria Group's scientific basis for Swedish occupational standards.

This publication is the 30th in the series, and contains consensus reports approved by the Criteria Group from July, 2008 through June, 2009. The consensus reports in this and previous publications in the series are listed in the Appendix (page 116).

Johan Högberg
Chairman

Johan Montelius
Secretary

The Criteria Group has the following membership (as of June, 2009)

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Consensus Report for Molybdenum and Molybdenum Compounds

February 4, 2009

Information for this report was obtained from literature searches of Arbline, Cheminfo, Cisdoc, HSDB, Hseline, Medline, Mhidas, Nioshtic, Oshline, Rilosh, Riskline and Toxline in March of 2007. Supplementary searches of the Toxline (including Pubmed) and SPIN databases were made in January and July of 2008. The Criteria Group published a previous Consensus Report on molybdenum in 1983 (65).

Chemical and physical data

Substance formula	CAS No.	Mol weight	Melting point (°C)	Boiling point (°C)	Solubility in water
Molybdenum Mo	7439-98-7	95.95	2629	4612	Insoluble
Molybdenum disulfide MoS_2	1317-33-5	160.08	1185	450[a] in air[b]	Insoluble
Molybdenum dioxide MoO_2	18868-43-4	127.94	—	—	Insoluble
Molybdenum trioxide MoO_3	1313-27-5	143.95	795	1155[a]	1.07 g/l (18°C)[c] 0.49 g/l (28°C)[c]
Ammonium molybdate $(NH_4)_2MoO_4$	13106-76-8	196.03	—[b]	—	400 g/l (20°C) Hot water[b]
Ammonium heptamolybdate (ammonium paramolybdate) $(NH_4)_6Mo_7O_{24}$	12027-67-7	1163.79	—[b] tetrahydr.[d]: $-H_2O$, 90	— tetrahydr.: 190[b]	— tetrahydr.: 430 g/l Hot water[b]
Sodium molybdate Na_2MoO_4	7631-95-0	205.92	687	—	443 g/l (20°C)
Sodium molybdate, dihydrate $Na_2MoO_4 \cdot 2H_2O$	10102-40-6	241.95	$-2H_2O$, 100	—	562 g/l Cold water
Calcium molybdate $CaMoO_4$	7789-82-4	200.01	965	—	Insoluble Hot water[b]
Molybdenum pentachloride $MoCl_5$	10241-05-1	273.21	194	268	—[b]
Ammonium tetrathiomolybdate $(NH_4)2MoS_4$	15060-55-6	260.26	—	—	—

[a]sublimates [b]disintegrates [c]information differs [d]CAS No. 12054-85-2
Data in the table are from References 13, 14, 36, 67.

Molybdenum is either a silver-white metal (crystalline form) or a dark gray powder. It occurs in nature in various minerals, the most important of which is molybdenite (molybdenum disulfide). The most important oxidation states for molybdenum in compounds are +II, +III, +IV and +VI. Most molybdenum compounds are either insoluble or disintegrate on contact with water, but some molybdates, notably ammonium and sodium molybdate, dissolve readily (14, 28, 36, 67). Molybdenum pentachloride can react with water and damp air to form chlorine gas, and is reported to be caustic (http://nj.gov/health/eoh/rtkweb/documents/fs/1311.pdf). The molybdenum compounds most prevalent in work environments are molybdenum trioxide and molybdates (65). A total of 932 tons of molybdenum trioxide was used in Sweden in 2005. The substance was a registered ingredient in 44 products (SPIN database, http://195.215.251.229/DotNetNuke/default.aspx).

Occurrence, use

Molybdenum is produced from molybdenum trioxide. Most molybdenum is used in production of various types of steel and heat-resistant alloys for use in high-temperature environments. It occurs, for example, in the automobile and airplane industries, in machine manufacture, in the electrical industry and in welding. Molybdenum levels (total dust) of $\leq$2.3 $\mu g/m^3$ (personal monitors) and $\leq$4 $\mu g/m^3$ (stationary monitors) were measured around arc furnaces during production of stainless steel in 1999 (27). Molybdenum levels around 100 – 300 $\mu g/m^3$ (total dust) were measured during a workshift of welding in stainless steel (personal monitor, 13 samples, 30 minutes each; 1 subject) (50). Molybdenum levels of 0.2 – 18 $\mu g/m^3$ (total dust, 18 samples) were measured in the breathing zones of welders using coated electrodes for gas arc welding in mild steel and stainless steel during the 1970s (69, 70, 71). Much higher air concentrations of molybdenum have been measured in other contexts, however: 1.5 – 7.9 mg Mo/m^3 (total dust) was reported in the breathing zone of a worker grinding, cutting and heating metallic molybdenum (70 – 200 minute samples); the background level recorded by a stationary monitor (about 5 hours) was 0.7 mg Mo/m^3 (61). In a plant producing molybdenum oxides from molybdenum disulfide, air levels of molybdenum recorded by stationary monitors were in the range 3 – 33 mg/m^3 (total dust), with an 8-hour time-weighted average (TWA) of 9.5 mg Mo/m^3. The monitors indicated a respirable dust content of 1 – 4.5 mg Mo/m^3 (75).

Molybdenum is also used in production of glass and ceramics – in electrodes, stirrers, kiln components, pigments, enamelwork etc. Molybdenum trioxide and soluble molybdates (e.g. sodium molybdate, sodium molybdate dihydrate) are used as corrosion inhibitors. Molybdenum and molybdenum compounds (including molybdenum disulfide, molybdenum trioxide, molybdenum dioxide) are also important as catalysts in numerous industrial processes, especially in the oil and gas industries. Molybdenum oxides and molybdates may be added to plastics as flame retardants. Sodium molybdate is a reported ingredient in some de-icers. Other areas of use include leather preparation and chemical fertilizers.

Molybdenum disulfide has a special use as a lubricant (2, 14, 36, 65, 67; SPIN database, http://195.215.251.229/DotNetNuke/default.aspx).

Ammonium tetrathiomolybdate has been used therapeutically to counteract accumulation of copper in the body, e.g. with Wilson's disease. In recent years, tetrathiomolybdate (the ammonium salt and analogues) has also been proposed, and to some extent tried, for treating cancers and inflammatory diseases. Ammonium tetrathiomolybdate in oral doses (induction doses) of about 120 – 240 mg/day has been used in cancer therapy, for example (10, 12, 37, 67).

Molybdenum is an essential trace element for humans, animals and plants (2, 65). It is a component of several enzymes, including sulfite oxidase, which oxidates sulfite to sulfate and is necessary for the metabolism of amino acids containing sulfur, and xanthine oxidase (xanthine dehydrogenase), which is active in purine metabolism and oxidates hypoxanthine and xanthine to uric acid (44, 67, 75). The major dietary sources of molybdenum are grains, dairy products and vegetables, and estimated daily intake is on average about 0.1 – 0.2 mg. Plasma concentrations of molybdenum are usually in the range 0.3 – 1.1 µg/l, but can be as high as 2 – 4 µg/l if intake in food is extremely high (0.5 – 1.5 mg/day) (67).

Uptake, biotransformation, excretion

Molybdenum and its compounds can be absorbed in the digestive tract. Data on some of the insoluble compounds (e.g. molybdenum disulfide) indicate extremely low uptake, but the soluble molybdenum compounds are absorbed quite well. Animal data record uptakes of 40 – 85% for single doses of hexavalent molybdenum compounds (17, 67). Uptake via inhalation depends on solubility and particle size. Quantitative data are scarce, but experiments with guinea pigs, mice and rats indicate that hexavalent molybdenum compounds such as molybdenum trioxide are readily absorbed via inhalation (14, 17, 49, 67). An inhalation study with exposures to 6.7, 20 and 67 mg Mo/m^3 as trioxide reports that average blood concentrations of molybdenum were respectively about 800, 1800 and 6000 µg/l for male rats, 350, 650 and 2400 µg/l for female rats, 100, 210 and 770 µg/l for male mice, and 70, 200 and 520 µg/l for female mice (49). No noteworthy molybdenum uptake was seen in an older study in which guinea pigs were exposed to molybdenum as disulfide (dust) at levels around 285 mg Mo/m^3 (17). Plasma levels up to 365 µg Mo/l were reported in a study of workers exposed to molybdenum trioxide (and other substances), but it is not clear whether uptake was via lungs or via both lungs and digestive tract. The air level of molybdenum was 9.5 mg/m^3 (8-hour TWA, total dust) (75).

In the blood, molybdenum is bound as molybdate to red blood cells and plasma proteins. It is distributed rapidly to most tissues, including liver, kidneys and bones, although levels in fat tissue are low. Molybdenum can also pass the placental barrier (14, 67). The literature contains very little data on molybdenum metabolism, but it is known that molybdenum passes through the cell membrane as molybdate, and in the cell, in a copper-dependent process, forms a cofactor (Moco) that is subsequently incorporated into various enzymes, including xanthine

oxidase and sulfite oxidase. Inability to form this cofactor results in death. Sulfite oxidase is activated by addition of the cofactor, whereas activation of xanthine oxidase requires a further step (addition of sulfur) (44). The biological half time of molybdenum in humans has been reported to be on the order of weeks, and the substance is excreted as molybdates, mostly in urine (14). Molybdenum can also be excreted in breast milk (14). Studies in which sheep were given intravenous injections of radioactively labeled di-, tri- and tetrathiomolybdate indicate that the substances undergo a hydrolysis process of several steps leading to formation of molybdate, which is then excreted primarily in urine (41).

Tetrathiomolybdate given orally together with food forms complexes with the copper and proteins in the food in the digestive tract, thus severely impeding copper absorption. With oral intake between meals, tetrathiomolybdate is taken up into the blood and forms a complex with albumin and free copper in serum. This complex-bound copper is unavailable for cellular uptake (56). In a comparative study, oral doses of radioactively labeled copper (^{64}Cu) were given to rats that also received 12 ppm Mo in feed as either ammonium tetrathiomolybdate or sodium molybdate: copper uptake was greatly reduced by the tetrathiomolybdate but unaffected by the sodium molybdate. Distribution of the absorbed copper was also affected by the tetrathiomolybdate (45).

Toxicity

Human data

Molybdenum, copper and sulfate have complex interrelationships in the body. Exposure to molybdenum may disrupt the normal balance between molybdenum and copper, leading to copper deficiency – especially when sulfate intake is insufficient. The serum level of the copper-containing enzyme ceruloplasmin can be used as a measure of the body's copper status. The most prominent symptoms of severe copper deficiency are anemia, neutropenia and osteoporosis, and the first clinical indication of copper deficiency is a low blood count (usually anemia). High uric acid levels in serum due to increased activity of the enzyme xanthine oxidase have also been reported with elevated levels of molybdenum (10, 14, 53, 56, 65, 67, 75).

Therapeutic use of tetrathiomolybdate in cases of cancer (it inhibits vascularization) has side effects such as bone marrow suppression, anemia and/or leukopenia (10, 12, 53). Side effects of this type (as well as effects on the liver) have also been seen in treatment of Wilson's disease with tetrathiomolybdate (Table 1). In one study it is also reported that none of the patients had abnormal uric acid levels in serum (average doses of ammonium tetrathiomolybdate were 100 – 300 mg/day) (11).

There is a case report of acute poisoning due to intake of ammonium heptamolybdate (Table 1). The victim had drunk about half a spoonful of the substance stirred into her coffee, and immediately thereafter she had stomach cramps accompanied by violent, bloody vomiting and severe diarrhea. On arrival at

the hospital she was found to have severe gastritis. After a day or so, kidney effects (especially on renal tubuli) and moderate anemia were also seen. Two months later the monitored parameters (kidney damage, anemia) were completely normal. The authors suggest that the loss of blood may have contributed to the anemia (6).

An elevated prevalence of a gout-like condition has been reported in people living in a part of Armenia where the earth and vegetation contain high levels of molybdenum. Calculated daily molybdenum intake for the population in this area was 10 – 15 mg, compared to 1 – 2 mg Mo/day in a control area. Daily intake of copper was also somewhat lower in those with higher molybdenum exposure (5 – 10 mg vs. 10 – 15 mg). Elevated uric acid levels in serum correlated to elevated levels of molybdenum and to elevated activity of xanthine oxidase. Uric acid in serum from the sick subjects (n = 17) was on average 81 mg/l, compared to 53 mg/l in healthy subjects (n = 35) living in the same area and 38 mg/l in controls (n = 5) living in another area. The average level of copper in blood was somewhat (significantly) lower in subjects with symptoms. Enlarged livers, digestive disturbances and renal disease were also reported in the sick subjects, but no further details were given (Kovalskii *et al.* 1961, cited in References 14 and 75).

Acute inflammation in a metatarsal joint and moderately elevated serum urate (564 µmol/l; 95 mg/l) were seen in a 36-year-old industrial electrician exposed to molybdenum. He later developed pain in the shoulders, wrists and ankles (not rheumatic in origin). Serum urate gradually dropped to 482 µmol/l (81 mg/l) over the next two years, and during this period the patient's exposure to molybdenum also stopped. He had formerly been exposed while grinding, cutting and heating metallic molybdenum. Total dust measurements (70 – 200 minute samples) made during a reconstruction of his working conditions showed levels of 1.6 – 10 mg/m^3 (1.5 –7.9 mg Mo/m^3) in the breathing zone, and stationary monitoring (about 5 hours) showed a background dust level of 0.9 mg/m^3 (0.7 mg Mo/m^3). Three weeks after the reconstruction, the patient had another attack of acute arthritis in his ankle. Serum urate was at that time 484 µmol/l (81 mg/l) and he received a definite diagnosis of gout. According to the authors, a connection to occupational exposure to molybdenum can be suspected but not established (61).

In a study of 25 workers exposed to molybdenum, 18 reported on a questionnaire that they had experienced some type of health problem – joint pain, backache, unspecified changes in skin or hair, diarrhea etc. (see Table 1). It was reported that, although no evidence of molybdenum-induced gout could be seen in the responses, turnover among the workers was high. Blood profiles ("complete blood counts") were reported to be normal, and 20 of the 25 workers had normal results on lung function tests. Results for the control group (24 students) were not given in this part of the study. Elevated levels (sic!) of ceruloplasmin were reported in serum of the workers (average values: 50.5 mg/dl vs. 30.5 mg/dl in controls), and average levels of uric acid were 59 mg/l for the workers and 50 mg/l for the controls. Plasma levels of molybdenum were 9 – 365 µg/l in the workers and up to 34 µg/l in controls. No direct correlations were seen between

molybdenum, uric acid and ceruloplasmin in plasma/serum. The factory produced molybdenum oxides from molybdenum disulfide (ammonium dimolybdate, ammonium heptamolybdate, sodium molybdate and calcium molybdate were also reported to occur). Stationary monitors indicated air levels of molybdenum (in total dust) in the range 3 – 33 mg/m^3, and average exposure was calculated to be 9.5 mg Mo/m^3 (8-hour TWA). The molybdenum content in respirable dust (≤10 μm) from the stationary monitors was in the range 1 – 4.5 mg/m^3 (mostly soluble molybdenum oxides). A calculation based on a level of 1.02 mg Mo/m^3 in respirable dust indicated a daily body burden of 10.2 mg molybdenum from soluble molybdenum particles (75). The data reported in the study provide no indication of whether there was a correlation between molybdenum exposure and symptoms for any single subject, but normal levels of uric acid in serum and normal blood profiles argue in general against such a correlation.

Of 19 workers exposed to levels of 1 to 25 mg/m^3 metallic molybdenum and molybdenum trioxide for 4 to 7 years, 3 were reported to suffer from symptoms such as breathing difficulty and frequent coughing. Pneumoconiosis (early stages) was verified by lung x-rays (14). In a subsequent study, 43 workers with inhalation exposure to molybdenum trioxide were compared with 23 unexposed workers. Exposure was reported to consist of fine to ultra-fine (diameter <250 nm) dust, but no air levels were given. Respiratory symptoms (chest pains, breathlessness, coughing) lasting more than 6 weeks were reported by 33 of the exposed workers; the other 10 reported no symptoms. No clear indications of interstitial lung disease were seen on lung x-rays, but "discrete abnormalities" were observed in 29/33 (with symptoms) and 5/10 (symptom-free) exposed workers. Lung x-rays of controls were normal. Results on lung function tests were better for the exposed group than for controls. Cytologic examination of bronchoalveolar lavage fluid (BAL) indicated possible sub-clinical alveolitis in exposed workers with respiratory symptoms (54).

In a large study in which patch tests with several metal salts were administered, 2% molybdenum pentachloride yielded a positive result in 6/211 persons and 2% ammonium molybdate in 3/208 persons. One person tested positive for both substances. Three of the eight patients worked with metal (14). In a study with 80 volunteers, 2 showed sensitization when given a patch test with 1% molybdenum pentachloride in water (20). Allergic reactions were observed in 1/128 patients with hip replacements containing metal when they were patch tested with 1% ammonium molybdate in water, and in 4/131 patients with stainless steel stents when they were patch tested with 0.5% molybdenum pentachloride in vaseline (35, 68). Critical review of the data collected by German dermatology clinics in the 1992 – 1999 period shows positive results to patch tests with 1% ammonium heptamolybdate in water for 3/787 patients and also 7 uncertain/irritation reactions (14, 23).

Animal data

There are large inter-species differences in the toxic effects of molybdenum, and intakes of copper, molybdenum and sulfate have a complex interrelationship. Ruminants are regarded as particularly sensitive to high levels of molybdenum in diet, probably due to the formation of thiomolybdate in the sulfide-rich environment of the rumen (14, 36, 65, 75). Molybdenum poisoning can present the same symptoms as copper deficiency: anemia, diarrhea, weight loss, joint abnormalities, osteoporosis, reproductive problems, kidney damage etc. (4, 33, 48, 57, 67, 75).

Insoluble and soluble molybdenum compounds were compared in an older toxicity study with rats. There were no deaths or visible indications of toxicity in the rats given ≤500 mg Mo/day as molybdenum disulfide in feed for 44 days, but animals were clearly affected by repeated exposure to high doses of calcium molybdate, molybdenum trioxide and ammonium heptamolybdate tetrahydrate. With repeated exposures, the lethal doses for 50% of animals were calculated to be about 100 mg Mo/kg b.w./day for calcium molybdate, 125 mg Mo/kg b.w./day for molybdenum trioxide, and 333 mg Mo/kg b.w./day for ammonium heptamolybdate tetrahydrate (17). In another rat study, 4 weeks of exposure to equimolar concentrations (0.8 mmol/100 g feed; about 77 mg Mo/100 g feed) of sodium molybdate, molybdenum trioxide and molybdenum pentachloride resulted in greatly reduced weight gain and increased activity of alkalic phosphatases. The molybdate and trioxide were about equally toxic in these respects, the pentachloride somewhat less so. Potassium tetrathiomolybdate was much more toxic – all the animals were dead after 4 weeks (73). Thiomolybdate also yielded more obvious effects (diarrhea, deaths) than molybdate in a study in which guinea pigs were given either ammonium molybdate or thiomolybdate (mostly tetrathiomolybdate) in drinking water (260 µmol Mo/l; 25 mg Mo/l) before and during gestation (25). In a comparative study, rats were given 12 mg Mo/kg feed for 11 days: with administration as ammonium tetrathiomolybdate, weight gain was much poorer and there was total inhibition of ceruloplasmin activity in plasma, whereas the sodium molybdate had no such effects (46).

When feed containing 6 mg Mo/kg as ammonium tetrathiomolybdate was given to male rats for 5 weeks (about 0.6 mg Mo/kg b.w./day, assuming a feed intake of 100 g/kg b.w./day), the exposure resulted in anemia, diarrhea, skeletal damage, growth retardation and fur discoloration. The skeletal damage was also seen in 1/6 animals at a dose level of 4 mg Mo/kg feed (about 0.4 mg Mo/kg b.w./day). Copper content in the feed was relatively low in this study, however (3 mg/kg) (46). Poor growth was also observed in another rat study, in which sodium molybdate dihydrate was given in feed for several months. This effect was seen in males at 20 mg Mo/kg feed (about 2 mg Mo/kg b.w./day) and in females at 80 mg/kg feed (about 8 mg Mo/kg b.w./day). No anemia was seen in either dose group (both sexes) (29, 74). In a recent study in which 1, 4, or 12 mg ammonium tetrathiomolybdate/kg b.w./day (0.4, 1.5 or 4.5 mg Mo/kg b.w./day) was given by gavage to female rats for about a month (prior to and during early gestation) and to

male rats for 2 months, no clinical indications of toxicity are reported at any dose level. Significantly lower body weights and feed consumption, as well as mild anemia, were seen in male rats in the high-dose group, however (40). Effects on kidneys were given particular emphasis in another study in which male rats were given ammonium heptamolybdate tetrahydrate (40 or 80 mg Mo/kg b.w./day) by gavage for 8 weeks. Poorer growth with lower body weights ($p<0.001$) and somewhat lower kidney weights was observed at the high-dose level. Measurements of various parameters of kidney function indicated mild chronic renal failure in the high-dose group, with poor glomerular filtration and effects on distal tubuli (8).

In inhalation studies, guinea pigs were exposed to various molybdenum compounds 1 hour/day, 5 days/week for 5 weeks (17). At an average exposure of 286 mg Mo/m^3 as molybdenum disulfide dust, respiratory rate was increased during the exposure but there were no other reported indications of toxicity (1/25 animals died after 3 exposures). Analysis data showed no uptake of molybdenum disulfide. Exposure to 159 mg Mo/m^3 in the form of neutralized calcium molybdate yielded no clinical indications of toxicity, but 5/24 animals died during the experiment (pneumonia occurred, but it is not clear whether it was related to the exposure). Elevated molybdenum levels were found especially in the lungs, but also in bones and kidneys. Exposure to 205 mg Mo/m^3 in the form of molybdenum trioxide dust was extremely irritating (eyes, nostrils). Diarrhea, weight loss, ataxia and hair loss were also reported, as were changes in liver, spleen and lungs. Half of the animals died. There were elevated molybdenum levels in kidneys, bones, spleen and liver, and small amounts of molybdenum were also found in the lungs. With exposure to molybdenum trioxide in the form of smoke (53 or 191 mg Mo/m^3), however, only 1/25 animals died at the high exposure and no other indications of toxicity were observed. Analyses revealed low levels of molybdenum in all examined tissues, including lungs (2, 17).

In a modern inhalation study, rats and mice were exposed to molybdenum trioxide in air concentrations of 3 to 300 mg/m^3, 6 hours/day, 5 days/week for 14 days. Significantly lower weights were noted at 100 mg/m^3 (male rats only) and 300 mg/m^3 (both species, both sexes), but there were no clinical indications of toxicity (49). Mice and rats were exposed for 13 weeks to molybdenum trioxide dust in air concentrations of 1 to 100 mg/m^3. There were no significant effects on body weights or organ weights and no clinical indications of toxicity, and no significant exposure-related differences were seen in histopathologic, hematologic and clinical-chemical examinations. There were, however, significantly elevated copper levels in livers of the mice (females at 30 mg/m^3, both sexes at 100 mg/m^3). Rats and mice exposed to 10, 30 or 100 mg molybdenum trioxide/m^3 (about 6.7, 20 or 67 mg Mo/m^3) for 2 years showed no symptoms typical of molybdenum poisoning (diarrhea, anemia) or toxicologically significant differences in bone density or “bending” of the femur, but histopathologic examination revealed exposure-related changes in respiratory passages. The rats had elevated incidences of chronic alveolar inflammation at the two higher

exposure levels (very mild to moderate; both severity and incidence increased with dose). Higher incidences of hyaline degeneration of respiratory and olfactory epithelium and squamous cell metaplasias in epiglottal epithelium were also seen at all exposure levels (see Table 2), but these changes were regarded as non-specific defense mechanisms/adaptations. The mice showed similar slight changes in nose and larynx (non-specific or indications of defense/adaptation), but had no chronic inflammation in alveoli (49).

No immunotoxic effects were observed in mice after 14 days of exposure to 1 – 100 ppm (primary antibody response) or 1 – 25 ppm (phagocyte activity and sub-populations of lymphocytes in spleen, lymph nodes and peripheral blood) molybdenum pentachloride in feed. The sensitization potential of molybdenum pentachloride as a contact allergen was also tested in a modified Local Lymph Node Assay (LLNA) (1). Although the authors report that the assay showed molybdenum pentachloride to be a weak, non-specific contact irritant, the only definite conclusion that can be drawn from this experiment is that, under these experimental conditions, molybdenum pentachloride has a weaker sensitizing potential than the strong experimental contact allergen oxazolone (positive control). Molybdenum pentachloride was ranked a potent contact allergen in the Guinea Pig Maximization Test (GPMT) (17/20 positive animals vs. 3/20 controls), whereas sodium molybdate pentahydrate was not (7). Sodium molybdate has been reported to cause primary irritation. A 20% solution caused reddening of conjunctiva. Calcium molybdate produced no skin irritation and no significant eye irritatation when tested on rabbits. No further details on these studies are given (36).

Tetrathiomolybdate has been shown in animal models to have an anti-inflammatory effect – it provides protection against doxorubicin-induced heart damage, for example. Strong inhibition of the inflammatory cytokines TNFα and IL-1β and inhibition of IL-2, an immune-regulatory cytokine, have been reported. Tetrathiomolybdate has also been reported to provide protection against bleomycin-induced pulmonary fibrosis and against liver damage from certain substances, including carbon tetrachloride (24).

A study in which rat liver cells were exposed *in vitro* to nanoparticles (30 nm or 150 nm) of molybdenum trioxide (24 hours) yielded evidence of cytotoxicity, expressed as leakage of lactate dehydrogenase and decline in mitochondrial function, at 250 µg/ml but not at ≤100 µg/ml (both particle sizes) (26).

Mutagenicity, genotoxicity

Molybdenum trioxide was not mutagenic to *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 or TA1537, either with or without metabolic activation (49, 76). Negative results with molybdenum trioxide are also reported in *in vitro* rec assays with *B. subtilis* (31, 32). Nor was there any increase in incidence of sister chromatid exchange (SCE) or chromosome aberrations in Chinese hamster ovary (CHO) cells *in vitro*, either with or without metabolic activation (49). In a micronuclei test (250 – 750 µg/ml) and in a cell trans-

formation test (50 – 200 µg/ml) on Syrian hamster embryo (SHE) cells *in vitro,* however, molybdenum trioxide yielded positive results at the higher dose levels (19, 34). Matthews *et al.* (42) report molybdenum trioxide to be inactive in a cell transformation test on BALB/c-3T3 cells (2.3 – 11 mM).

Ammonium heptamolybdate was mutagenic to *E. coli* (2 – 10 mM) and slightly to moderately genotoxic to *B. subtilis* (rec assay) in *in vitro* tests (31, 32, 47). There is, however, a briefly described study reporting that ammonium heptamolybdate hexahydrate is not mutagenic in bacteria tests (*S. typhimurium* and *E. coli*) (3). In another type of bacteria test (prophage induction in the microscreen assay), which measures DNA damage, sodium molybdate was weakly positive without metabolic activation (59), and a co-mutagenic effect was reported in an *in vitro* study with *E. coli* (elevated numbers of mutants induced by UV light at concentrations ≥100 µM) (58). However, sodium molybdate was not genotoxic in SOS-response chromotests with *E. coli* when tested without metabolic activation (52). Further, sodium molybdate dihydrate was judged to be inactive in a bacterial bioluminescence test (72). In a test on *Saccharomyces cerevisiae*, sodium molybdate (40 – 150 mM) had effects on cell division (meiosis), expressed as a dose-dependent increase of diploid spores (63). In another study on yeast, no mutations or other genetic changes (gene conversions) were observed in tests with sodium molybdate and ammonium molybdate (62). In tests for micronuclei induction in human lymphocytes *in vitro,* both ammonium heptamolybdate tetrahydrate (0.1 – 2 mM) and sodium molybdate monohydrate (0.1 – 5 mM) were positive (66).

Molybdenum pentachloride was reported to be negative in *in vitro* bacterial tests for genotoxicity (*B. subtilis,* rec assay; *E. coli*, SOS-response chromotest) (47, 52).

Molybdenum disulfide was reported to be negative in rec assays with *B. subtilis* (31, 32) and positive in an *in vitro* cell transformation test (SA7/SHE) (62 – 1000 µg/ml) (22).

There are few *in vivo* studies. Mutagenic effect on gametes was studied in a dominant lethal test. Sodium molybdate monohydrate was given to male mice (200 or 400 mg/kg b.w./day, 2 days, intraperitoneal injection) that were then mated with untreated females over a two-month period. Dose-dependent increases of post-implantation losses (10.6%, 16.3%, vs. 6.7% in controls) were observed, and the effect was most pronounced the first week after the injections (66). In the same study, sodium molybdate monohydrate was judged to be weakly genotoxic in the micronuclei test on mice after intraperitoneal injection for two days (200 or 400 mg/kg b.w.) (66). Molybdenum trichloride was found to be genotoxic in an *in vivo* test with *Drosophila* (10 – 50 mM *per os* during the larval stage) (51).

In summary, molybdenum compounds at high concentrations have been reported to be weakly mutagenic in some, but not all, bacteria tests. Some results from *in vitro* tests with mammalian cells were negative, but micronuclei tests with, for example, hamster cells and human lymphocytes, yielded positive results

at high concentrations. Weakly positive results were also seen in an *in vivo* micronuclei test and a dominant-lethal test with mice.

An English summary of an older Russian study (5) states that significantly higher frequencies of chromosome changes ("rearrangements") in peripheral lymphocytes were observed in cytogenetic examinations of workers exposed to molybdenum and molybdenum compounds, when compared with controls. A closer examination of the Russian text reveals that cells from 47 persons exposed to molybdenum, molybdenite or ammonium paramolybdate, or to ammonium paramolybdate and molybdenum trioxide, and 23 controls were analyzed and that the deviations noted in the exposed subjects were usually of the chromatid type (not numerical or structural chromosome changes). The study reports that air levels (monitoring times not given) were 1.5 –10.2 mg/m^3 for molybdenum, 0,9 – 8.4 mg/m^3 (usually 3.6 – 6.2 mg/m^3) for ammonium paramolybdate, <23.5 mg/m^3 for molybdenum trioxide, and <54 mg/m^3 for molybdenite.

Carcinogenicity

In a hospital-based case-control study (15) with 478 cases of lung cancer and 536 controls, cases and controls were interviewed on occupation, job duties and exposures (self-estimated), smoking habits and hobbies. Exposures to 16 different proven or suspected lung carcinogens were classified using a job/exposure matrix (yes/no, duration), but the study contains no data on air concentrations or blood levels. It was found that for those who, according to this classification, had at some time been occupationally exposed to molybdenum, the risk of lung cancer was doubled (Odds Ratio: 2.1; 95% Confidence Interval: 1.2 – 3.7, based on 52 exposed cases and 34 exposed controls). The analyses were adjusted for smoking habits, socioeconomic factors and education. When the cancer cases were divided into thirds according to length of employment and compared with unexposed, the greatest risk increase was seen in the highest third (>21 years; OR: 3.3; 95% CI: 1.3 – 8.3). For the middle and lower thirds the odds ratios were moderately but not significantly elevated (1.8 and 1.6 respectively). It is rather remarkable that, despite the relatively high exposure prevalences, no risk increases were seen for exposure to established lung carcinogens such as asbestos (161 exposed cases, 169 exposed controls) or PAH (235 exposed cases, 233 exposed controls). This may be due to low exposure levels, but no assessment can be made since intensity of exposure was not classified in the study. This study is difficult to evaluate, since the participation rate is not reported and no effects are reported for established lung carcinogens despite the fact that such exposures were relatively common. Further, the proportion of subjects classified as occupationally exposed to molybdenum was remarkably high (86 of a total of 1014 study participants).

In an NTP cancer study (49), F344/N rats and $B6C3F_1$ mice were exposed to molybdenum trioxide levels of 10, 30, or 100 mg/m^3 (corresponding to 6.7, 20 or 67 mg Mo/m^3), 6 hours/day, 5 days/week for 2 years (Table 2). The incidences of alveolar/bronchiolar adenoma or carcinoma increased in the male rats with a marginally significant positive trend, but was on the same order of magnitude as

in historic controls. For male mice, there were significantly elevated incidences of alveolar/bronchiolar carcinoma at all dose levels (16/50 at 10 mg/m^3, 14/49 at 30 mg/m^3, 10/50 at 100 mg/m^3 vs. 2/50 in controls). The combined incidences of alveolar/bronchiolar adenoma and carcinoma were significantly elevated at the two lower dose levels (27/50, 21/49 vs. 11/50 in controls). Female mice had significantly higher incidences of alveolar/bronchiolar adenoma at 30 and 100 mg/m^3 (8/49, 9/49 vs. 1/50 in controls) and adenoma/carcinoma combined at 100 mg/m^3 (15/49 vs. 3/50 in controls). According to the NTP, the study provides "some evidence" that molybdenum trioxide is carcinogenic to male and female mice, "equivocal evidence" of carcinogenic activity in male rats, and "no evidence" of carcinogenic activity in female rats.

A significant increase of lung adenomas was reported in mice after intraperitoneal injections (3 times/week) of the maximum tolerable dose of molybdenum trioxide in a sodium chloride solution (19 injections; total dose 4.75 g/kg b.w.) and the substance was judged to be weakly carcinogenic. At lower total doses (0.95 and 2.74 g/kg b.w.) the substance did not induce a significant elevation in lung tumor incidence (64). The value of this type of experimental study, however, has been questioned (65).

Reduced tumor incidence has been reported in laboratory animals receiving various carcinogenic nitroso compounds in drinking water/feed along with sodium molybdate. The protective effect of molybdenum is attributed mostly to increased detoxification by denitrosation. There may also be some anti-carcinogenic effect due to the increased urinary excretion of copper caused by molybdenum, which reduces copper levels in serum (49). Experiments with tetrathiomolybdate (which binds copper) indicate, however, that the enzyme superoxide dismutase 1 (SOD1), which contains copper, may be inhibited within endothelial cells: vascularization and cell proliferation may thus be inhibited before a systemic reduction of copper levels can be detected. In tumor cells, intracellular inhibition of SOD1 can result in inhibited cell proliferation as well as cell death (30).

The International Agency for Research on Cancer (IARC) has published no carcinogenicity classifications for molybdenum or molybdenum compounds.

Effects on reproduction

Human studies

Correlations between semen quality and levels of various metals in blood are examined in a recently published study (43). The subjects were 219 men who, together with their partners, had sought treatment at two infertility clinics. Among the men there were both normal and deviant semen findings, since fertility problems can be due to either partner or both. The semen parameters measured were volume, number, concentration, proportion of motile sperm, and morphology. Reference values, e.g. a sperm concentration of 20 million/ml, were used for classification (greater than/less than). Blood samples were analyzed for molybdenum, arsenic, cadmium, chromium, copper, lead, manganese, mercury,

selenium, thallium and zinc, and on the basis of blood levels the subjects were divided into at least 3 groups for each metal in order to investigate dose-dependent relationships with the studied semen parameters. The detection limit for molybdenum in blood was 1.0 µg/l, and only 30% of the samples had levels above this. Persons with molybdenum levels below the detection limit constituted the low-exposure group (reference group), and persons with detectable levels of molybdenum were divided into two groups of equal size: medium exposure (70 – 85th percentile) and high exposure (>85th percentile). The highest blood level of molybdenum measured in the study was 5.4 µg/l. Several different statistical strategies were used, and the analyses included smoking habits, age and presence of other metals in blood. The study reports significant or suggestive associations and dose-dependent trends for elevated molybdenum levels in blood and elevated risk of sub-normal sperm concentration and sperm morphology, whereas the relationships between other metals and semen quality were less consistent. For example, for molybdenum the adjusted odds ratios for sperm concentration were 1.4 (95% CI: 0.5 – 3.7) for the medium-exposure group and 3.5 (95% CI: 1.1 – 11) for the high-exposure group, and for morphology were 0.8 (95% CI: 0.3 – 1.9) and 2.6 (95% CI: 1.0 – 7.0) respectively. Interaction between molybdenum and low blood levels of copper or zinc were also indicated in the study, but with broad confidence intervals. The authors state in conclusion that more and larger epidemiologic studies, as well as mechanistic studies, are needed to confirm their results, and point out some weaknesses in their study, e.g. that only one blood sample and one semen sample was taken from each participant and that only a small proportion of the blood samples contained detectable amounts of molybdenum. They also point out that blood molybdenum levels in the general population may show considerable geographic variation (43). No analyses of exposure conditions were made, although dietary factors were discussed. The possibility that, for example, copper deficiency or endogenous metabolic changes might be able to explain the variation in molybdenum levels was not discussed. The relevance of this study to assessment of molybdenum is unclear.

Animal studies

Rats and mice were exposed to 10, 30 or 100 mg molybdenum trioxide/m^3 (6.7, 20 or 67 mg Mo/m^3) 6.5 hours/day, 5 days/week for 13 weeks: no significant effects on sperm count or sperm motility were reported in either species. Exposure to molybdenum trioxide for 2 years on the same schedule caused no definite exposure-related changes visible in histopathologic examination of reproductive organs (e.g. testes, epididymis, prostate, seminal vesicles, uterus, ovaries). The blood levels of molybdenum (mean values) in the 2-year study with molybdenum trioxide were reported to be about 220, 800, 1800 and 6000 µg/l in male rats and 60, 350, 650 and 2,400 µg/l in female rats, at 0, 6.7, 20 and 67 mg Mo/m^3 respectively. Molybdenum levels in the blood of the mice at these exposures were much lower (49).

Effects on the fertility of male rats were examined in a study in which the animals were given 10, 30, or 50 mg sodium molybdate/kg b.w. (about 4.7, 14 or 23.3 mg Mo/kg b.w.) by gavage 5 days/week for 60 days. Observations at the two higher dose levels included degenerative changes in testes, reduced sperm counts, lower sperm motility and higher proportions of abnormal sperm. Reduced fertility, increases in pre- and post-implantation losses, reduced numbers of living fetuses and lower values for weight and length of fetuses were also noted (examined at 14 mg Mo/kg b.w., mating with untreated females) (55). In an older study, infertility was reported in male rats given sodium molybdate dihydrate for several months after weaning at a dose level of 80 mg Mo/kg feed, but not at 20 mg Mo/kg feed (about 8 or 2 mg Mo/kg b.w./day, assuming a feed intake of 100 g/kg b.w./day). Histologic examination of testes from infertile animals revealed degenerative changes (29, 74).

Ammonium tetrathiomolybdate was given to male rats by gavage in doses of 1, 4 or 12 mg/kg b.w./day (0.4, 1.5 or 4.5 mg Mo/kg b.w./day) for 2 months. The NOEL in this study for both systemic effects and reproduction effects was 1.5 mg Mo/kg b.w./day (significant reductions of ceruloplasmin in serum were seen at all dose levels, however). Effects observed at the high-dose level included mild anemia, histopathological changes in testes and epididymis, lower sperm counts, greatly reduced sperm motility, and <9% morphologically normal sperm. However, no effects on reproductive function, expressed as gravid females, were noted at any dose after the males had been treated for 4 weeks (40).

Sodium molybdate dihydrate was given to female rats in drinking water (5, 10, 50 or 100 mg Mo/l) from weaning until day 21 of gestation (2 to 3 months): estrous cycles were prolonged in dose groups receiving ≥10 mg Mo/l (≥1.6 mg Mo/kg b.w./day, assuming a weight of 100 g per rat) but fertility was unaffected. After mating with untreated males, gravid females in the groups given water containing ≥10 mg Mo/l had more resorptions and somewhat less developed fetuses (histologic examination). At levels above 10 mg Mo/l there were also smaller fetuses and significant reductions in fetal weight. No significant effects (estrous cycle, embryotoxicity, fetal development) were seen at the lowest dose level (0.9 mg Mo/kg b.w./day, the NOAEL in this study). The feed was reported to contain adequate amounts of copper (18, 74). Effects were observed in a three-generation reproduction study in which mice were maintained on drinking water containing soluble molybdate (greatest effects in the F_3 generation) at the same level (10 mg Mo/l, about 1.5 mg Mo/kg b.w./day, assuming intake of 150 ml water/kg b.w./day). There was a higher number of runts in the F_3 generation (11/123 vs. 0/230 in controls) and several pairs in this generation were sterile. Early postnatal deaths were more frequent in both the F_1 generation (15/238 vs. 0/209 in controls) and the F_3 generation (34/123 vs. 1/230 in controls), and in the F_2 generation there were 5 dead litters (0 in controls) (60, 74).

Guinea pigs given ammonium molybdate in drinking water (260 µmol Mo/l; 25 mg Mo/l) prior to and during gestation showed no effects on estrous cycle, and there were 10/12 live births in exposed animals vs. 21/23 in controls (4/8 females

in the exposed group did not become gravid). In the same study, there were only 3/37 live births to guinea pigs receiving the same amount of molybdenum (260 µmol Mo/l) in the form of thiomolybdate (mostly tetrathiomolybdate) prior to and during gestation or during gestation only. At a dose level of 130 µmol Mo/l (12.5 mg Mo/l) as thiomolybdate there were 10/21 live births in guinea pigs exposed prior to and during gestation, and 18/19 live births in those exposed during gestation only. Dose-dependent effects, including diarrhea and deaths, were observed in mothers in the thiomolybdate-treated groups, but estrous cycles were unaffected (25).

Ammonium tetrathiomolybdate in doses of 1, 4 or 12 mg/kg b.w./day (0.4, 1.5 or 4.5 mg Mo/kg b.w./day) was given to female rats by gavage for up to 1 month (until day 6 of gestation): there were no clinical indications of toxicity and no significant effects on studied parameters (including estrous cycle, mating, fertility, implantations, resorptions, number of fetuses) (40). These authors report in an abstract that no evidence of embryotoxicity, fetotoxicity or teratogenicity was seen in rats after daily oral administration of the substance on days 6 – 17 of gestation, at dose levels of 2 or 6 mg/kg b.w./day, whereas 20 mg/kg b.w./day (7.4 mg Mo/kg b.w./day) increased the number of resorptions (38). Another abstract reports results from an experiment with rabbits: oral administration of 6, 20 or 60 mg ammonium tetrathiomolybdate (2.2, 7.4 or 22 mg Mo)/kg b.w./day on days 7 – 20 of gestation yielded significant reductions in red blood cell counts (mothers), more resorptions, and spontaneous abortions in 50% of animals in the high-dose group (one animal in the medium-dose group also aborted). Dose-dependent increases of a “carpal/tarsal flexure” malformation were also seen in the two higher dose groups (7.4 and 22 mg Mo/kg b.w./day) (39).

Sheep given repeated injections of ammonium tetrathiomolybdate (1.7 mg/kg b.w. intravenous or 3.4 mg/kg b.w. subcutaneous) after copper poisoning recovered their health, but developed endocrine disturbances and fertility problems. There were elevated levels of molybdenum especially in pituitary and adrenals, where pathological changes were also observed (pituitary atrophy or degeneration with depletion of ACTH, LH and FSH; adrenal cortex atrophy). Testicular atrophy (reduced spermatogenesis) and ovarian degeneration were also observed. The authors suggest that thiomolybdate probably binds to copper in the pituitary/hypothalamus and thus inhibits activity of a copper-dependent enzyme that is central to bioactivation of peptide hormones (e.g. pituitary hormones), which can lead to pathological changes in reproductive organs and impaired reproductive ability (21).

Nanoparticles (30 nm) of molybdenum trioxide have been tested for cytotoxicity on mouse spermatogonia *in vitro* (5 – 100 µg/ml; 48 hours). No distinct effects on cell morphology were observed under a phase-contrast microscope, but apoptosis tests indicated increased apoptosis at concentrations >25 µg/ml. Reduced mitochondrial function was noted at concentrations ≥50 µg/ml, and increased leakage of lactate dehydrogenase was observed at levels as low as 5 µg/ml. No significant effects on cellular metabolic activity (mito-

chondrial function) or membrane function (lactate dehydrogenase leakage) were observed in a similar test with soluble sodium molybdate (9).

Dose-effect/dose-response relationships

Available data on humans support no estimates of dose-response or dose-effect relationships for occupational exposure to metallic molybdenum or the molybdenum compounds treated in this report. Use of ammonium tetrathiomolybdate to treat cases of cancer (induction doses corresponding to about 0.6 – 1.3 mg Mo/kg b.w./day) has side effects associated with copper deficiency, most notably in the form of anemia and/or leukopenia. Side effects of this type, as well as effects on the liver, have also been seen in treatment of patients with Wilson's disease (Table 1).

Molybdenum poisoning in animals has symptoms similar to those of copper deficiency: anemia, diarrhea, weight loss, joint abnormalities, osteoporosis, reproduction problems and kidney damage (4, 33, 48, 57, 67, 75). Intakes of copper, molybdenum and sulfate have a complex interrelationship, however, and there are large inter-species differences in sensitivity. Ruminants are regarded as particularly sensitive to high levels of molybdenum in diet because of the thiomolybdate formed in the rumen (14, 36, 65, 75). Some studies in which rats, mice and guinea pigs were given relatively low oral doses of tetrathiomolybdate or soluble molybdate are summarized below. There are only a few inhalation studies with laboratory animals, but available data are summarized below and in Table 2.

Tetrathiomolybdate – per os

Anemia, diarrhea, skeletal damage, impaired growth and hair discoloration were reported in male rats given ammonium tetrathiomolybdate in diet at dose levels equivalent to about 0.4 – 0.6 mg Mo/kg b.w./day (4 – 6 mg Mo/kg feed) (46). In this study, however, the copper content in the feed was quite low (3 mg/kg). In a more recent study in which ammonium tetrathiomolybdate was given by gavage, the NOEL for systemic and reproduction effects in male rats was about 1.5 mg Mo/kg b.w./day and the LOEL was about 4.5 mg Mo/kg b.w./day (lower body weight, mild anemia, effects on testes and sperm). For the female rats, the NOEL for systemic and reproduction toxicity was 4.5 mg Mo/kg b.w./day (40). The LOEL for reproduction toxicity (resorptions, developmental defects) in female rats and rabbits is reported in two abstracts to correspond to 7.4 mg Mo/kg b.w./day (38, 39). In a study with guinea pigs, drinking water containing 130 or 260 µmol Mo/l (12.5 or 25 mg Mo/l) as thiomolybdate (mostly tetrathiomolybdate) had dose-dependent effects on mothers and on reproduction (25).

Soluble molybdate – per os

Lengthened estrous cycles, but no effects on fertility, were seen in female rats given drinking water containing sodium molybdate dihydrate in doses corresponding to ≥1.6 mg Mo/kg b.w./day (≥10 mg Mo/l). Increases in resorptions

and somewhat less developed fetuses were seen at the same levels. The NOAEL in the study can be given as 0.9 mg Mo/kg b.w./day (18, 74). Effects on reproduction (early postnatal deaths, unsuccessful reproduction) were also observed in mice given about 1.5 mg Mo/kg b.w./day (10 mg Mo/l) as soluble molybdate in drinking water for three generations (60, 74). In a study with administration of sodium molybdate dihydrate in feed, there was poorer growth in male rats at doses of about 2 mg Mo/kg b.w./day (20 mg Mo/kg feed) and in female rats at about 8 mg Mo/kg b.w./day (80 mg Mo/kg feed). This concentration also caused infertility and degenerative changes in testes in the male rats (29, 74). In a more recent study, no significant effects on testes/sperm were observed after sodium molybdate was given to male rats by gavage in doses corresponding to 4.7 mg Mo/kg b.w./day. Effects of this nature, as well as poor fertility and reproduction, were observed at about 14 mg Mo/kg b.w./day (55).

Molybdenum trioxide – inhalation

Long-term inhalation exposure to molybdenum trioxide at levels ≥10 mg/m^3 (≥6.7 mg Mo/m^3) resulted in elevated incidences of lung tumors in mice, but there was no observable dose-response relationship. Dose-dependent, very mild to moderate inflammatory changes were seen in lungs of rats after long-term exposure to ≥30 mg/m^3 (≥20 mg Mo/m^3) (49). No symptoms indicative of molybdenum poisoning (e.g. anemia, hair loss, diarrhea) were observed in either mice or rats at air levels ≤100 mg/m^3 (≤67 mg Mo/m^3). Effects on body weight, but no clinical indications of toxicity, were seen in mice and rats after short-term exposure to 300 mg/m^3 (200 mg Mo/m^3) (49). An older study, however, reports severe irritation, diarrhea, weight loss, ataxia, hair loss and death of 50% of animals (guinea pigs) at about the same air levels (about 200 mg Mo/m^3) (17).

Molybdenum sulfide – inhalation

Elevated respiratory rates were observed in guinea pigs during exposure, but no other indications of toxicity were reported in an older study with short-term exposure to molybdenum sulfide at an air level of about 290 mg Mo/m^3 (17).

Conclusions

Data are altogether too sparse to allow determination of a critical effect for occupational exposure to molybdenum or the molybdenum compounds treated here. The most prominent side effects of therapeutic administration of tetrathiomolybdate are anemia and/or leukopenia.

Animal data indicate that the easily soluble molybdenum compounds, including molybdenum trioxide, are more toxic than the less soluble ones. Effects on reproduction have been seen in some studies in which mice and rats were given soluble molybdate in drinking water at daily doses corresponding to about 1.5 mg Mo/kg b.w. In one study, lung tumors were seen in mice after long-term inhalation exposure to molybdenum trioxide – in the males at exposures as low as 10 mg/m^3

(6.7 mg Mo/m^3). This may be regarded as some evidence that molybdenum trioxide is carcinogenic to male and female mice. Evidence of carcinogenic activity in male rats was equivocal, and there was no evidence of carcinogenic activity in female rats.

Table 1. Observations in humans in connection to exposure to molybdenium compounds.

Type of exposure Substance	Exposure	Calculated dose[a]	Observations	Ref.
Occupational: molybdenite, molybdenum oxides, soluble molybdates	Stationary: 1-4.5 mg Mo/m^3 respirable dust; 9.5 mg Mo/m^3 total dust (8-h TWA)	0.15 mg Mo/kg b.w./day[b]	Normal blood profiles, normal serum urate (various symptoms with unclear relevance).	75
As cancer treatment: ammonium tetrathiomolybdate	120-240 mg/day[c] *per os*	0.6-1.3 mg Mo/kg b.w./day[c]	Side effects[d]: primarily anemia and/or leukopenia.	10, 12
As treatment for Wilson's disease[e]: ammonium tetrathiomolybdate	100-300 mg/day[f] *per os*	0.5-1.6 mg Mo/kg.b.w./day[f]	Normal serum urate. Side effects: anemia, leukopenia, thrombocytopenia, elevated ASAT, ALAT and alkalic phosphatases.	11
Poisoning: ammonium heptamolybdate (case report)	About half a spoonful of powder (in coffee)	60-180 mg Mo/kg b.w.[g]	Stomach pain, bloody vomit, severe diarrhea, renal effects, anemia.	6

[a] Assuming 100% uptake with both oral administration and inhalation exposure, and body weight of 70 kg (unless other information is provided).
[b] Based on the body burden calculated by the authors: 10.2 mg Mo/day (1.02 mg Mo/m^3 as respirable dust, 10 m^3 inhaled air).
[c] Induction doses
[d] Relatively rare when serum ceruloplasmin levels are 10 - 15 mg/dl.
[e] The disease is characterized by copper accumulation in the liver, liver damage, brain damage and low ceruloplasmin in blood (16, 44).
[f] Average doses
[g] Assuming that the density of ammonium heptamolybdate is the same as that of ammonium heptamolybdate tetrahydrate, 2.5 g/ml (13), half a spoonful is 2.5 – 7.5 ml; and further assuming a body weight of 60 kg.

Table 2. Exposure-effect correlations observed in laboratory animals exposed by inhalation to some inorganic molybdenum compounds.

Exposure		mg Mo/m^3	Species	Effects	Ref.
MoO_3					
3 mg/m^3	6.5 hrs/day, 5 days/wk, 13 weeks	2	Rat Mouse	No significant effects on body or organ weights or in histopathologic, hematologic or clinical-chemical examination; no clinical indications of toxicity; no effect on sperm.	49
10 mg/m^3	6.5 hrs/day, 5 days/wk, 13 weeks	6.7	Rat Mouse	Same as above.	49
10 mg/m^3 (MMAD* 1.5 μm)	6 hrs/day, 5 days/wk, 2 years	6.7	Rat	Elevated incidence of very mild non-neoplastic changes in respiratory passages (squamous cell metaplasia in epiglottal epithelium and, only in females, hyaline degeneration of nasal respiratory and olfactory epithelium).	49
10 mg/m^3 (MMAD* 1.3 μm)	6 hrs/day, 5 days/wk, 2 years	6.7	Mouse	*Males only:* Elevated incidence of lung tumors: alveolar/bronchiolar carcinoma (16/50 vs. 2/50), alveolar/bronchiolar adenoma/carcinoma (27/50 vs. 11/50). *Both sexes:* Elevated incidence of very mild, non-neoplastic changes in respiratory passages (squamous cell metaplasia in epiglottal epithelium, metaplasia in alveolar epithelium).	49
30 mg/m^3	6.5 hrs/day, 5 days/wk, 13 weeks	20	Rat	No significant effects on body or organ weights or in histopathologic, hematologic or clinical-chemical examination; no clinical indications of toxicity, no effects on sperm.	49
30 mg/m^3	6.5 hrs/day, 5 days/wk, 13 weeks	20	Mouse	Significantly elevated copper levels in liver (females). No other significant effects on body or organ weights or in histopathologic, hematologic or clinical-chemical examination; no clinical indications of toxicity, no effect on sperm.	49
30 mg/m^3 (MMAD* 1.6 μm)	6 hrs/day, 5 days/wk, 2 years	20	Rat	Higher incidences of very mild to moderate non-neoplastic changes in respiratory passages (hyaline degeneration of nasal respiratory epithelium, squamous cell metaplasia in epiglottal epitheliium, chronic alveolar inflammation and, only in females, hyaline degeneration of olfactory epithelium).	49
30 mg/m^3 (MMAD* 1.4 μm)	6 hrs/day, 5 days/wk, 2 years	20	Mouse	Elevated incidence of very mild non-neoplastic changes in respiratory passages (squamous cell metaplasia in epiglottal epithelium, metaplasia in alveolar epithelium). Higher incidence of lung tumors: *males*: alveolar/bronchiolar carcinoma (14/49 vs. 2/50), alveolar/bronchiolar adenoma/carcinoma (21/49 vs. 11/50); *females:* alveolar/bronchiolar adenoma (8/49 vs. 1/50).	49

Table 2. Cont.

Exposure		mg Mo/m^3	Species	Effects	Ref.
As smoke	1 hr/day, 5 days/wk, 5 weeks	53	Guinea pig	No indications of toxicity.	2, 17
100 mg/m^3	6.5 hrs/day, 5 days/wk, 13 weeks	67	Rat	No significant effects on body or organ weights or in histopathologic, hemataologic or clinical-chemical examination; no clinical indications of toxicity.	49
100 mg/m^3	6.5 hrs/day, 5 days/wk, 13 weeks	67	Mouse	Significant elevation of copper content in livers. No other significant effects on body or organ weights or in histopathologic, hemataologic or clinical-chemical examination; no clinical indications of toxicity.	49
100 mg/m^3 (MMAD* 1.7 μm)	6 h/day, 5 days/wk, 2 years	67	Rat	*Males only:* Higher incidence of lung tumors: alveolar/bronchiolar adenoma/carcinoma (4/50** vs. 0/50). *Both sexes:* Higher incidence of mild to moderate non-neoplastic changes in respiratory passages (hyaline degeneration of nasal respiratory epithelium, squamous cell metaplasia in epiglottal epithelium, chronic alveolar inflammation. *Females only*: Hyaline degeneration of olfactory epithelium.	49
100 mg/m^3 (MMAD* 1.5 μm)	6 hrs/day, 5 days/wk, 2 years	67	Mouse	Higher incidence of lung tumors: *males:* alveolar/bronchiolar carcinoma (10/50 vs. 2/50); f*emales:* alveolar/ bronchiolar adenoma/carcinoma (15/49 vs. 3/50), alveolar/bronchiolar adenoma (9/49 vs. 1/50). *Both sexes:* Higher incidence of very mild to mild non-neoplastic changes in respiratory passages (hyaline degeneration of nasal respiratory epithelium, hyperplasia in laryngeal epithelium, squamous cell metaplasia in epiglottal epithelium, metaplasia in alveolar epithelium. *Females only:* Hyaline degeneration of olfactory epithelium.	49
as smoke	1 hr/day, 5 days/wk, 5 weeks	191	Guinea pig	No indications of toxicity. One animal died.	2, 17
	1 hr/day, 5 days/wk, 5 weeks	205	Guinea pig	Extremely irritating to eyes and nostrils; diarrhea, weight loss, ataxia and hair loss; changes in liver (vacuolization, necrotic foci), spleen and lungs (exudate); 26/51 animals died.	2, 17

Table 2. Cont.

Exposure		mg Mo/m^3	Species	Effects	Ref.
$CaMoO_4$ (neutralized)					
	1 hr/day, 5 days/wk, 5 weeks	159	Guinea pig	No clinical indication of toxicity; 5/24 animals died (pneumonia was reported, but not clear whether it was related to exposure).	2, 17
MoS_2					
	1 hr/day, 5 days/wk, 5 weeks	286	Guinea pig	Elevated respiratory rate during exposure but no other indications of toxicity; 1/25 animals died.	2, 17

* Mean value for aerodynamic particle diameter.
** On the same order of magnitude as historic controls.

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Consensus Report for Grain Dust

February 4, 2009

This report is a scientific up-date of the Consensus Report for Grain Dust (46) by the Swedish Criteria Group which was based mainly on a document produced jointly by the Swedish Criteria Group and NIOSH, U.S.A (10). Relevant studies up to the end of year 2008 have been considered for inclusion.

Composition of grain dust

Grain dust consists of 60 to 80% organic material and 20 to 40% inorganic material. The major components of grain dust are fragments of cereal grain such as fractured grain kernels and weed seeds and husks. Other important components include micro-organisms (bacteria and moulds), storage mites, insects and insect parts, hair, feathers and excrement from rodents and birds, residues of pesticides, herbicides, fertilizers, silica and dirt (16). Microbes and components of microbes, especially endotoxin, are particularly important in farming (34, 58). The composition of grain varies depending on several factors. Important factors include type of grain, geographic site, wetness of the season, storage temperature of the various components. Several constituents of the grain dust, for instance the microbial composition, are dependent on growing conditions, season, humidity, temperature, storage conditions etc. (16).

Exposure

Exposure to grain dust occurs in farming, processing of animal feed, grain mill work and in shipment and storage of grain. In Sweden, total dust concentrations over 50 mg/m^3 have been measured in receiving pits for grain with the hatches closed and fans running (63). Personal monitors worn by stevedores unloading grain recorded averages of 5.6 to 9.5 mg/m^3 total dust over a work-shift, but peaks of 66 mg/m^3 were noted (68). In 1993-94, studies using parallel measurements of total dust and inhalable dust were conducted in 17 industries associated with grain exposure by the Swedish National Board of Occupational Safety and Health (2), Table 1. Measurements were made in a variety of different tasks in farming, animal feed operations, milling and elevator work. Personal samplers were used. The previously used sampling method for total dust, the Millipore method with cassettes having a filter diameter of 25 mm and 37 mm, respectively, was compared with the IOM-sampling method for inhalable dust. In farming, exposure to grain inhalable dust occurred mostly for short periods with high mean exposure concentrations of 9.7 mg/m^3 measured typically in threshing and drying operations. In work with animal feed, the mean exposures over a working

Table 1. Total and inhalable grain dust concentrations in farming, animal feed work, milling and grain elevator work in Sweden 1993-1994 and relation between total dust and inhalable dust measurements. Modified from ref. (2).

Type of work or work place	Total dust (mg/m^3)		Inhalable dust (mg/m^3)		Total dust/ inhalable dust (regression coefficient)
	Highest value	Average (range)	Highest value	Average (range)	
Farming	6.6	5.5 [a]	11.6	9.7 [a]	0.84 [g]
Animal feed work	11.7	2.7 (1.6-4.3) [b]	27.9	11.1 (5.5-18.0) [b]	0.34 [h]
Mills	20.6	3.3 (0.5-10.7) [c]	38.2	7.3 (1.2-21.4) [e]	0.57 [h] 0.42 [g] 0.22 [h,i]
Grain elevators	5.8	1.9 (0.7-4.8) [d]	21.7	4.9 (0.9-12.8) [f]	0.66 [g]

[a] Based on one 3-hours measurement, the rest of the day there was no exposure.
[b] Based on 6 full day measurements at 3 companies.
[c] Based on 17 full day measurements at 6 companies.
[d] Based on 6 full day measurements at 5 companies.
[e] Based on 16 full day measurements at 6 companies.
[f] Based on 7 full day measurements at 6 companies.
[g] Total dust measured with 25 mm cassette.
[h] Total dust measured with 37 mm cassette.
[i] Sweeping and sifting.

day ranged from 5.5 to 18.0 mg/m^3 (mean 11.1 mg/m^3, inhalable dust). High concentrations were measured in maintenance tasks. The corresponding exposures in mills and grain elevator work were 1.2-21.4 (7.3) and 0.9-12.8 (4.9) mg/m^3, respectively. However, in grain elevator work, the production on the sampling day was low, and the concentrations may underestimate those of a normal production day. Tasks performed from a control room were associated with low concentrations. The results are summarised in Table 1. For further details of exposure levels for different work tasks within the different work environments with grain dust exposure, see ref. (2). Endotoxins were also analysed in 8 grain dust samples from 4 mills. Endotoxin levels varied between 10 and 971 ng/m^3. There was no correlation between dust levels and endotoxin levels (2).

The studies by the Swedish National Board of Occupational Safety and Health (2) revealed that the IOM sampling method (inhalable dust), which conforms with the European standard EN 481:1993 and the corresponding Swedish standard SS-EN 481, measures significantly higher grain concentrations than the Millipore method (total dust), the difference being in most cases linear. However, the relations depend on the kind of grain dust and the diameter of the Millipore cassette used. The Millipore method with a cassette filter diameter of 37 mm measured significantly higher dust concentrations than the 25 mm filter diameter.

Table 1 shows the relations (regression coefficients) between total dust and inhalable dust, obtained in farming, animal feed work, milling and grain elevator work. The differences are important when comparing older measurements of total dust with the present ones of inhalable dust.

Uptake, biotransformation, excretion

There are no data on uptake of grain dust or its deposition in the lungs.

Toxic effects

The major health effects of inhaled grain dust are irritant symptoms from the respiratory tract, asthma, acute and chronic reduction of lung function that is non-asthmatic, and grain fever (16, 24, 27, 28). Apart from grain dust, endotoxins have been suggested as the cause of respiratory effects (34, 38, 57, 58). The mechanisms of the acute and chronic effects on the respiratory tract are largely unknown. Both grain dust and endotoxin can induce inflammatory response as shown by release of several interleukins, neutrophil chemotactic factor and as increases in neutrophils in association with decreased FEV_1 (18, 39, 66). Acid stress has been reported to be involved in the acute effects on the lung function, whereas oxidative stress is part of the regulation of chronic effects on lung function (23). Grain fever and asthma, being separate disorders, have here been reviewed under headings of their own. Studies on grain fever having a benign prognosis, and asthma, do not contribute to the recommendation of an exposure limit. The subdivision causes some overlapping in the text. In addition, there are a few historical case reports on pneumoconiosis in dock workers who handled mostly grain (30), allergic alveolitis (hypersensitivity pneumonitis, "farmer's lung") in farmers (21, 51) and serious dyspnea in grain workers (56).

Grain fever

Grain fever is an acute inhalation fever that also has been classified as organic dust toxic syndrome (ODTS) (54). The prevalence of grain fever in various studies has varied from 6% to 32% of exposed workers (16, 24, 61). Grain fever is characterized by chilliness and fever often accompanied by headache, malaise, myalgia, chest tightness, dyspnea, cough and expectoration. During a symptomatic phase leucocytosis with more immature white cells in peripheral blood is commonly seen. The radiograph is as a rule normal (26). When six grain workers and six controls were experimentally exposed to high doses of grain dust, grain fever symptoms were experienced by all exposed. Thus, grain fever is not thought to be induced by a specific immunological mechanism. It has been suggested that grain fever is caused by exposure to endotoxin (26).

Asthma

Sensitization to various constituents of grain dust occurs. In most cases of asthma among grain workers, however, the causative agent remains unidentified. IgE-mediated asthma has been reported to be caused by sensitization to wheat (25) and storage mites such as Glycycophagus destructor (20, 36) and Lepidoglyphus destructor (62). Sensitization among asthmatics to this particular storage mite occurred in 12% of the subjects and 4% of the asthmatics displayed sensitization to another storage mite, Acarus siro, as well. Studies on sensitization to grain dust among exposed workers have revealed IgE responses as shown by skin prick testing and determinations of serum specific IgE (50). The prevalence of allergic asthma among grain workers appears to be rather low. Grain exposure causes also non-allergic asthma. Endotoxin has been implicated as a cause of the non-allergic asthmatic reactions (3). A prevalence study of asthma among grain workers including 669 grain workers and 560 office workers was undertaken in Vancouver (14). The prevalence of current asthma was only 2.4% and 2.7%, respectively. As the prevalence of atopy was only 17.3% among grain exposed, and 31.3% among office workers, respectively, it was postulated that asthmatics are likely to move from grain dust exposure, which undoubtedly will cause aggravation of any asthma, occupational or non-occupational. Another indication of selection is the finding that wheeze, e.g. Senthilselvan *et al*. (55); Peelen *et al*. (52), is one of the most prevalent respiratory symptom among grain workers and is also significantly more prevalent among grain exposed than controls, whereas asthma appears less frequent and not in the same way more prevalent among exposed than among unexposed.

Acute and chronic effects on the respiratory system and eyes

Respiratory and eye symptoms

Early cross-sectional studies by doPico *et al.* (24, 27, 28) on grain workers in Minnesota, USA and by Broder *et al.* (5, 7, 8, 9) and Corey *et al.* (19) in Thunder Bay, Canada have demonstrated the high prevalence of respiratory symptoms among grain workers.

In the Minnesota studies, exposure levels were very high (range of means 10.3-253 mg/m^3), with 66% of measurements in excess of 15 mg/m^3. Consequently, the prevalences of symptoms were also high with 88.6% of the exposed grain handlers complaining of one ore more respiratory symptoms. Typical symptoms included cough, expectoration, wheezing, dyspnea, chest tightness, nasal stuffiness and eye irritation (24).

In a subsequent Minnesota study, grain workers were compared with unexposed city service workers (28). Lung function parameters were included (FEV_1, FVC, FEV_{25-75}, FEV_{50}, diffusion capacity). Respiratory symptoms and airflow obstruction (decline in FEV_1/FVC, FEV_{25-75} and FEV_{50}) were significantly more prevalent among exposed. It was also reported that the probability of having respiratory symptoms, especially chronic bronchitis and wheezing at work, are

independent of, and usually greater than, the probability of developing these symptoms from smoking.

Studying symptoms in 283 grain workers and 192 city service workers in relation to exposure (27), significantly higher prevalences of cough (48% vs. 32%), expectoration (38% vs. 19%), wheezing (13% vs. 9%), dyspnea (12 vs 6%), nasal stuffiness (38 vs. 26%) and eye irritation (13% vs. 6%) were found among grain workers. Of the 209 total dust measurements, 93% were below 10 mg/m^3 and 86% below 5 mg/m^3 with mean concentrations of 3.3 ±7.0 mg/m^3. At exposures >5 mg/m^3, the increases in the prevalences of cough, expectoration and dyspnea were highly significant.

In a follow-up study (9) conducted in 1980 on 441 grain elevator workers and 180 civic workers having participated in the original survey in 1977 (5) in Thunder Bay, Canada. Still available were 315 grain and 107 civic workers; the remainder had retired or left. The results of the 1980 study were compared with those of the 1977 study. Spirometry was obtained (FEV_1, FVC, FEF_{50}, FEF_{75}). Dust exposure was measured using a dust collector for respirable dust with a nylon cyclone and a cassette with a filter having a pore size of 0.8 µm. The respirable dust was the fraction collected on the filter, whereas the non-respirable fraction was collected in the cyclone (19). In 1977 the exposure was 0.4-1.6 for respirable dust and 3.6-36.8 mg/m^3 for non respirable dust. In 1980 this had dropped to 0.2-1.4 and 0.1-2.0 mg/m^3, respectively. Data of those workers who had participated in the 1977 study but left before the 1980 study were compared with those workers who had remained in work and were re-examined in 1980. The workers who had left were significantly younger, had a shorter duration of employment, a greater prevalence of reported eye irritation, cough, phlegm and shortness of breath than those who had remained in work. Civic workers who had left, while being likewise younger and had a shorter duration of employment than still employed, displayed no statistically significant differences in symptom prevalences. In 1980 there were little differences among currently exposed and controls; however, that result should be interpreted with caution due to the obvious healthy worker effect (9). In the Thunder Bay studies, exposure was measured as the respirable and the non-respirable fraction. The sum of respirable and non-respirable may be roughly compared with inhalable dust, although it is likely to be lower than inhalable dust. The study revealed a strong selection among grain exposed.

In 1978 and 1979, 47 newly employed grain handlers were studied immediately before or soon after starting their employment and re-examined two and a half months later. As controls, 21 civic workers were examined in a similar way (8). Exposure data were not given. Compared with the civic workers, grain handlers showed a remarkable increase in the prevalence of cough, phlegm and eye irritation between the two examinations. Consistent small decreases in lung function parameters (FEV_1, FVC, TLC and flow rates) were recorded, the decline in FEV_1 being the only statistically significant change. Changes were similar in smokers and non-smokers. In another study on Thunder Bay workers temporarily

laid off for a few months, the respiratory symptoms were partly reversible, whereas symptoms returned after the workers re-entered the former work (6).

More recent prevalences of respiratory symptoms were presented in a comparison between Dutch and Canadian grain handling industries (52). The Dutch animal feed studies comprised 315 workers from two animal feed elevators and 12 production factories (57) and 78 workers from the same factories tested four years later (60). The Dutch transfer elevator was represented by 438 workers. The exposure in the Dutch animal feed and transfer elevator industries was measured as inhalable dust (57, 60). The Canadian grain industry was represented by 339 workers employed in five terminal elevators (35) and by 67 dock workers (22). The exposure of the Canadian terminal elevator and dock workers was measured as total dust (35). A comparison between the two sampling methods revealed that the Dutch sampling device sampled roughly twice the amount of the Canadian device. Cumulative exposures were calculated using exposure data in different job categories. When interpreting the high frequency of symptoms among Canadian dock workers, despite low current and cumulative exposure levels, differences in sampling methods should be taken into account (Table 2). Canadian dock workers were not exposed on a daily basis and they were exposed to high peak exposures which varied over the day (Table 2). The Dutch workers

Table 2. Prevalences of respiratory symptoms among in Dutch and Canadian grain workers (52).

Symptoms and exposure	Dutch animal feed mills N =390 (%) [a]	Dutch transfer elevator N = 438 (%) [a]	Canadian terminal elevator N = 339 (%) [b]	Canadian dock workers N = 67 (%) [c]
Chronic cough	8	11	14	15
Chronic phlegm	4	10	19	24
Breathlessness	5	10	17	6
Ever wheeze	15	24	18	30
Frequent wheeze	5	12	6	9
Current exposure mean (SD) inhalable* or total** dust, mg/m^3	7.9 (9.6)*	44.6 (23.7)*	3.5 (2.0)**	2.0 (0.8)**
Cumulative exposure mean (SD) total dust, mg x year/m^3	108.5 (127.3)	643.6 (406.9)	157.6 (134.1)	22.3 (26.7)

[a] Smid *et al.* (57), Tielemans *et al.* (60).
[b] Huy *et al.* (35).
[c] Dimich-Ward *et al.* (22).

symptoms displayed a dose-response relationship. It can be noted that "ever wheeze" was the most common symptom among the Dutch animal feed and transfer elevator as well as Canadian dock workers. This comparative study demonstrates similar types and frequencies of symptoms related to grain exposure in two different countries (52).

Acute effects on lung function

A proportion of grain exposed workers are known to have a cross-shift decline in lung function. The reported cross-shift fall in FEV_1 varies in different studies (12, 27). The decrease has been significantly greater than in unexposed controls (19). Six men handling barley were followed for two working days by measuring flow volume curves every two hours. All six experienced falls in ventilatory capacity of up to 800 ml. A similar decrease in ventilatory capacity was seen in five male volunteers not previously exposed to barley dust, when they sat in a silo for two hours. Decreases in ventilatory capacity ranging from 200 ml to 800 ml were measured with recovery taking up to 72 hours (47). In a study including respiratory symptoms and lung function conducted in 283 grain workers and 192 city service workers exposed to a mean total dust level of 3.3 ±7.0 mg/m^3, cross-shift decreases in FEV_1, FVC, $Vmax_{50}$ and $Vmax_{75}$ were reported (27).

Similar decreases in FEV_1 were reported in previously unexposed individuals when exposed to high levels of grain dust indicating that the decline in lung function is not mediated by a specific immunological mechanism. The decrease of FEV_1 was unrelated to skin reactivity to grain dust antigens (27, 31, 47).

Asthmatic subjects appeared to have a greater decrease in lung function parameters across the shift than non-asthmatic workers. The authors (33) of the study suggested that this may be linked to an increased bronchial reactivity. The airflow obstruction seems to be unrelated to atopy (4). However, in another study no associations between cross-shift decline in FEV_1 and bronchial hyper-responsiveness or eosinophilia were found (32).

Endotoxin may be one causative toxic substance as experimental challenges tests with corn dust extracts and equivalent concentrations of endotoxin resulted in a similar degree of airflow obstruction (17, 37).

Inhalation challenge tests with grain dust have been accompanied by neutrophilia in the respiratory tract both in experimental animals (39) and in humans (66). Release of neutrophil chemotactic factor and IL-1 from macrophages is further indications of a non-allergic inflammatory reaction (44, 66). In experimental studies on the mechanisms of the neutrophil recruitment, grain dust extracts of grain sorghum, corn, oats, and soybeans were evaluated for their ability to directly attract human neutrophils using a blindwell neutrophil chemotaxis assay. Each extract was found to possess significant chemotactic activity causing cleavage of the complement proteins C3 and properdin factor B (PFB). The extracts also lead to the generation of C5a, a potent neutrophil chemoattractant generated by complement activation (65).

In an inhalation challenge cross-over study, inhalation challenge tests were performed with a buffered saline and aqueous corn dust extract to 15 non-smoking, non-asthmatic, non-atopic male grain handlers. Compared with buffered saline, inhalation of corn dust extract resulted in significant airflow obstruction, which was observed within 30 min of exposure and persisted for 5 h. Corn dust extract resulted in an acute inflammatory response characterized by higher concentrations of neutrophils, IL-1, IL-6, IL-8 , and TNF-alpha in bronchoalveolar lavage (BAL) fluid. The interleukin changes were also significantly associated with the decrease in FEV_1 (18).

Grain workers in the Port of Vancouver, Canada, were studied in a series of five studies (11, 12, 13, 31, 59). Relevant exposure data of the Port of Vancouver studies are given in Table 4. In one of the studies (12), no significant differences were noted between grain workers (610 subjects) and municipal employees (136 subjects) with regard to eye, nose and chest symptoms, though FEV_1 and FVC values were lower in the grain workers. However, in a sub-group of 33 grain workers (11), 22 had respiratory symptoms and reduced lung function. Of these, 11 showed hyper-responsiveness in a metacholine test, while the other 11 had symptoms indicating chronic bronchitis. None of them reacted to a skin test for grain dust.

Chronic effects

Enarson *et al.* (31) reported a 6-year prospective study on a cohort consisting of all grain handlers employed in four grain elevators in Canada. The study was conducted in 1975-1981 (11, 59). In order to identify the most important factors influencing lung function, a nested case-control was formed of 81 workers. Cases (27 workers) were defined as 10% of workers having shown the worst trend in FEV_1 over the study period, whereas referents were those 54 workers with the best trend. Each case was matched with two controls for age and smoking. The mean decline in FEV_1 in the exposed group was greater than 100 ml/year. There was a significant relationship between the odds ratio of being a case and the mean level of dust exposure at work. Thus, the risk of a decline greater than 100 ml/year was associated with a mean total dust level of >5 mg/m^3. The risk was not related to atopy, history of asthma, or presence of respiratory symptoms (31).

Another study (29) determined whether chronic bronchitis and pulmonary function abnormalities were more common in lifetime non-smoking grain elevator workers (n=90) than in lifetime non-smoking unexposed community control subjects (n=90). Lung function was assessed by spirometry and flow-volume curves. The prevalence of chronic bronchitis, defined as daily production of phlegm for 3 months/yr for at least 2 years, was higher in exposed workers (23.1%) than in non-smoking controls (3.3%) ($p < 0.01$). Also airflow obstruction (maximal mid-expiratory flow rate and maximal expiratory flow at 50% of VC) was significantly more prevalent in exposed non-smokers than in controls. No data on exposure levels were given.

The original health survey conducted in Thunder Bay, Saskatchevan, Canada in 1977 and reported by Broder *et al.* (5), compared over 441 grain elevator workers with a control group of nearly 179 municipal employees. In a subsequent study by Corey *et al.* (19), ventilatory performance was studied. Information on symptoms were acquired by a questionnaire. Spirometry (FEV_1, FVC, FEF_{50}, FEF_{25}, TLC, RV) was performed daily during a work week to a sub-group consisting of 47 grain workers and 15 municipal employees (19). On an average, the non-respirable dust concentration was 5.7 ±10.9 mg/m^3 and the respirable fraction 0.9 ±0.7 mg/m^3. The frequencies of coughing and positive skin reactions to grain dust were higher in grain workers than in controls. The FEV_1, FVC and FEV_1/FVC decreased from Monday to Wednesday and the decreases were sustained on Friday. Fifty percent of the workers had a daily reduction of at least 923 ml/second in FEF_{50} and 310 ml/sec in FEF_{25} for each 1 mg/m^3 increase in respirable dust concentration. The FEV_1 and FVC also dropped significantly more in grain workers, both over the work day and over the work week (19).

In the Port of Vancouver studies, elevator workers were surveyed every third year from 1975 to 1988. Air sampling using personal samplers was undertaken for measurement of exposure to total grain dust between the years 1974 and 1989. Altogether 781 personal air samples representing 20 job titles has been analysed as total dust over the 16 years. Workers included in the study by Huy *et al.* (35) were tested in the fifth survey in 1988, when detailed individual job histories were taken. Complete test results were available for 498 grain workers and 55 civic workers serving as an unexposed control group. Longitudinal analyses were performed on 385 grain workers and 52 controls for whom test results were available from at least one earlier survey. Respiratory symptoms included in the questionnaire were chronic cough, chronic phlegm production, wheezing and dyspnea. The spirometric parameters used for analysis were forced expiratory volumes in one second (FEV_1), forced vital capacity (FVC), and maximal mid-expiratory flow rate (MMEF). Matching exposure data with data on grain exposure, four exposure categories were defined: unexposed, <4, 4-9, >9 mg/m^3 (total dust). Estimated cumulative exposures were calculated for each worker. There were no differences between exposure groups with respect to reported use of dust masks, frequency of peak exposures or duration since last peak exposure. Exposure levels had declined over the years ranging in 1974 from 1.4 to 97.8 mg/m^3 and in 1989 from 0.1 to 79.6 mg/m^3.

Huy *et al.* (35) reported that phlegm production was statistically significantly more prevalent in all exposure groups, also in the low exposure group (<4 mg/m^3) than in the control group. The medium and high exposure groups had significantly higher rates of dyspnea than the controls. The study clearly demonstrated a dose-response relationship of respiratory symptoms, which was evident for all studied respiratory symptoms. The increase in symptoms was more pronounced in non-smokers and former smokers than among smokers.

A distinct dose-response between exposure categories and lung function parameters was demonstrated by Huy *et al.* (35). In the cross-sectional analyses

of the 1988 survey participants, FEV_1 and FVC were lowest in the highest exposure group. In the group with intermediate exposure (4-9 mg/m^3), a significantly reduced FEV_1 and lower levels of FVC and MMF were seen compared with the low exposure group (<4 mg/m^3) as well as the controls. FVC was significantly lower ($p<0.05$) in the low exposure group (<4 mg/m^3) compared with controls (5.04 l and 5.26 l, respectively) and still lower in the intermediate exposure group (4-9 mg/m^3), i.e. 4.88 l ($p<0.01$). In the longitudinal analysis, there was also a greater decline in FVC in the low-exposure group than among controls. A likewise greater longitudinal decline in FEV_1 and MMEF in the intermediate exposure group when compared with the low exposure group was reported. However, looking at the annual loss of lung function, the low exposure group was loosing lung function slower than the controls. The authors have commented this suggesting that this may be due to self-selection out of the exposed work by such workers who rapidly loose lung function (35).

As the results on lung function may be due to the higher exposure levels prior to 1978, a further comparison between workers in the low and intermediate exposure categories were undertaken including only workers employed since 1978, after which grain concentrations have been <10 mg/m^3. In the analysis using multiple linear regression coefficients for grain dust exposure level and duration, the result were identical to those seen for the entire population, i.e. lower lung function levels cross-sectionally and faster rates of decline in lung function longitudinally. Therefore, the authors did not consider the effects to be due to earlier higher levels of exposure. Analyzing the relationship between symptoms and lung function, dyspnea was found to be related to lower levels of FEV_1 and FVC (35).

Kennedy and co-workers (40) conducted a further study on the Port of Vancouver employees to study whether, and to what extent the decrease in lung function showed reversibility after retirement, and to assess whether the lung function impairment in former grain workers, if not reversible, also caused functional disability. The study comprised 82 retired grain workers and 54 retired civic workers, all of whom had participated in the Port of Vancouver grain elevator programme, thus having had lung function measurements every 3 year since 1975 as reported previously by Huy *et al.* (35) and Chan-Yeung *et al.* (15). In addition to spirometry, methods included questionnaires on respiratory symptoms as well as on the impact of breathing difficulties on the activities of daily life, chest radiography, and a 6-min walk test. Grain workers had still more dyspnea (44% vs. 11%) and lower FEV_1 (78.6 vs. 88.2% of predicted) and FVC (90.0 vs. 97.7% of predicted) values compared to the civic workers. There had been no reversibility of the lung function after retirement. The grain exposed walked a shorter distance and scored higher on the impairment of activities scale. Thus, the respiratory impairment of grain workers was associated with interference in the daily activities.

The Port of Vancouver studies have also revealed a relationship between cross-shift decreases in FEV_1 and future degree of decline in lung function. The decline in lung function at follow-up among highly exposed (>9 mg/m^3) workers was

greater in workers who had exhibited a cross-shift decrease. In general, the acute cross-shift decline in lung function was associated with a greater decline in lung function than in workers without an acute response to grain dust (41).

In a study in Saskatchewan, Canada, grain elevator workers were followed for 15 years, from 1978 to 1993 (49). Data on respiratory symptoms and spirometry (FEV_1, FVC) were obtained every three years. The 3-year periods were referred to as cycles. To predict the annual decline in FEV_1 and FVC, a transitional model using the generalized estimating equations (GEE) methodology (45). Exposure data were not included in this study. The study showed that previous FEV_1 was a significant predictor of future FEV_1 obtained in cycles II, III and V regardless of smoking status. Similarly, previous FVC was a predictor of FVC in cycles II, III and IV. The decline in FEV_1 and FVC was greater in cycles II and III than in cycles IV and V. The smaller decline in FEV_1 and FVC in cycles IV and V was considered to be due to dust control improvements (49).

In a cross-sectional study from the Netherlands of 194 male workers exposed to organic dust for a mean time of 16.3 years at grain elevators in five animal feed mills, Jorna *et al.* (38) measured lung function in relation to four exposure categories representing arithmetic means (unexposed, 0-4, 4-9, >9 mg/m^3). The controls comprised 55 non-production workers rarely exposed to grain dust, although some of them had previously been exposed. The exposure was characterized by the 50% cut-off diameter of 30 µm (corresponding fairly well with the inspirable, i.e. the inhalable, dust fraction) and endotoxin levels with a range of arithmetic means between 0.4-29.1 ng/m^3. Present exposure was based on 54 measurements of inhalable dust and endotoxin, whereas the cumulative exposure was derived from the personal job histories. In addition to spirometric flow volume curves, impedance measurement were performed. The analyzed impedance parameters were resistance at 8 Hz (R8) and 28 Hz (R28), as well as the difference between R28 and R8 (frequency dependence, FD), the reactance at 8 Hz (X8) and the resonant frequency (f_0).

The results reported by Jorna *et al.* (38) included: in multiple linear regression analysis, chronic bronchitis and "wheezing ever" were statistically more common among all exposure groups, including the low one (0-4 mg/ m^3) and displayed a clear dose-response relationship. Chronic bronchitis was also associated with cumulative dust exposure, whereas shortness of breath was related to present endotoxin exposure. Wheezing was related to both cumulative dust exposure and endotoxin exposure. Air flow decreased with increasing exposure in a dose-response manner. The mean FEV_1, PEF (peak expiratory flow), MMEF (maximal mid-expiratory flow) and FEF_{75} (forced expiratory flow at 75% of the expired vital capacity) decreased statistically significantly with increasing exposure at all levels when adjusted for age, height and smoking. Also the impedance parameters indicated an obstructive air flow. R8 and f_0 increased whereas X8 decreased with increasing exposure. In the linear regression analyses, FEV_1, PEF, MMEF, FEF_{75}, R8, X8, FD and f_0 all differed statistically signifycantly between the low exposure group (0-4 mg/m^3) and the unexposed, Table 3. Symptoms in this study were

Table 3. Mean values of lung function in control workers and production workers classified into exposure categories with respect to airborne grain dust (38).

Lung function	Controls n = 54 Mean (±SEM)	0-4 mg/m^3 § n = 97 Mean (±SEM)	4-9 mg/m^3 n = 17 Mean (±SEM)	9 mg/m^3 n = 25 Mean (±SEM)
FVC (l)	5.63 (0.12)	5.34 (0.08)	5.37 (0.20)	5.28 (0.21)
FEV_1 (l)	4.51 (0.11)	4.20 (0.08) *	4.10 (0.08) *	3.99 (0.20) ***
PEF (l/s)	12.3 (0.24)	11.5 (0.18) *	10.7 (0.35) **	10.4 (0.32) ***
MMEF (l/s)	4.45 (0.21)	3.80 (0.13) **	3.52 (0.40) **	3.30 (0.29) ***
FEF_{75} (l/s)	9.54 (0.34)	8.74 (0.22) **	8.03 (0.61) **	7.38 (0.49) ***
R8	2.23 (0.09)	2.52 (0.09) **	2.54 (0.28) **	3.50 (0.34) ***
X8	-0.03 (0.03)	-0.08 (0.04) **	-0.24 (0.17) **	-0.50 (0.16) ***
FD	0.29 (0.04)	0.16 (0.06) *	0.07 (0.13) **	-0.43 (0.20) ***
F_0	8.46 (0.20)	9.21 (0.35) *	10.9 (1.47) **	14.1 (1.48) ***

§ Exposure categorization by arithmetic mean. Statistical testing by linear regression adjusted for age, height, weight and smoking.
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

shown to be associated with decreased lung function. Thus, symptoms compatible with chronic bronchitis were significantly associated with a decrease in FEV_1 and MMEF. Also shortness of breath was related to changes in FEV_1. All changes in lung function parameters were more closely associated with endotoxin exposure than to inhalable dust exposure (38).

Post *et al.* (53) reported from the Netherlands a five-year follow-up study on 140 grain elevator workers that were included in a previous study by Smid *et al.* (57). Exposure was calculated using eight-hour inhalable dust samples taken in eight different facilities. Categories of exposure were >10, 4-10 and <4 mg/m^3. The corresponding endotoxin exposure categories were >40, 20-40 and <20 ng/m^3, respectively (53). The logistic regression analysis showed that workers with a dust exposure >4 mg/m^3 (or endotoxin >20 ng/m^3) at the survey conducted in 1986-88 had at the follow-up examination higher risk of rapid decline in FEV_1 (OR 3.3; 95%CI 1.02-10.3) compared with the low exposure (<4 mg/m^3) group. Adverse effects were not found in the low exposure group, i.e. exposed to <4 mg/m^3 inhalable dust. The presence of respiratory symptoms at the base-line examination was a strong predictor of subsequent loss to follow-up, whereas base-line lung function was not. The authors emphasized the need to take into account the obvious selection out of grain dust exposure during the study period when interpreting results (53).

In the comparison between Dutch and Canadian grain handling industries (presented in section Respiratory and eye symptoms and Table 2), a distinct dose-response relationship between cumulative exposures to grain dust and FEV_1 was

demonstrated (52). The dust exposures varied considerably between industries, the highest exposure being found in Dutch transfer elevator work (mean current 44.6 mg/m^3 and cumulative exposure 643 mg x year/m^3) and the lowest among Canadian dock workers (mean current 2.0 mg/m^3 and cumulative exposure 22.3 mg x year/m^3). However, sampling methods differed considerably between Dutch and Canadian measurement, the Canadian sampling method arriving at much lower concentrations than the Dutch method. A correction factor of two was suggested to even out the difference (52).

Mutagenicity

No information was available on mutagenic effects of grain dust.

Carcinogenicity

A few epidemiological studies on populations exposed to grain dust studies have been published. In Finland, all working persons born between 1906 and 1945 were followed during 1971-95. The follow-up comprised 30 million person-years. Exposures were estimated using the population census. A raised SIR of 3.55 (95%CI 3.0-7.72) was reported for laryngeal cancer among men, mainly grain millers, exposed to plant dusts. No associations between exposure and nasal cancer, lung cancer or mesotheliomas were found (43).

In a study on 1325 deceased members of the American Federation of Grain Millers´ life insurance plan, the proportionate mortality ratio (PMR) was raised for cancers of the lymphatic and hematopoietic systems (PMR 149, 95%CI 106-209). Grain mill workers had the highest risk for lymphatic and hematopoietic cancers (PMR = 202), particularly lymphosarcoma and reticulum cell sarcoma (PMR = 216) and other lymphatic neoplasms (PMR = 272); of the cancers in the latter category, three were myelomas (<1 expected) (1). An association between farming and multiple myeloma has been proposed in a series of meta-analyses comprising 32 peer-reviewed studies published between 1981 and 1996. A relative risk of 1.23 (95%CI 1.14-1.32) was found. The estimator of relative risk of female farmers was 1.23 (95%CI = 1.17-1.29). Exposures possibly contributing to the occurrence of myelomas include pesticides and infectious microorganisms (42). A similar increase in the incident of multiple myeloma was found in a cohort of 140 208 Swedish farmers; the incidence was increased also in parts of Sweden where the use of pesticides have been less frequent (64).

The studies on carcinogenicity deal with mixed, unspecific exposures. It is, therefore, not possible to draw any conclusion about carcinogenicity of grain dust.

Reproductive effects

No information was available on reproductive effects of grain dust.

Dose-response/dose-effect relationships

Relevant data on relationships between grain dust concentrations and observed effects have been compiled in Table 4.

Several studies have been consistent in reporting indications of a healthy worker effect in elevator work (9, 15, 48, 53, 57, 67). When interpreting data on dose-effect and dose-response relationships, it should be borne in mind that selection out of the exposed environment will lead to an underestimation of the risk of the remaining survival population. It will weaken any associations between exposure and decline in lung function and other health effects, especially in cross-sectional studies. Asthmatics are likely to leave the exposed work.

In long-term follow-up studies, respiratory symptoms and decline in lung function display an exposure-response relationship within exposure categories of <4; 4-9, >9 mg/m^3 inhalable dust (35, 38). Symptoms such as cough, phlegm, "chronic bronchitis" and wheeze were significantly more common among workers exposed to levels <4 mg/m^3 measured as inhalable dust (38), as well as total dust (35). Symptoms from the respiratory tract compatible with chronic bronchitis as well as shortness of breath were significantly associated with subsequent decreases in lung function (38).

Dose-response studies indicate that an excessive decline in forced vital capacity (FVC) occurs at concentrations <4 mg/m^3 measured as total dust (35), and in forced expiratory volyme (FEV_1) at concentrations <4 mg/m^3 measured as inhalable dust (38). The decline in lung function increases in an exposure-response manner within exposure categories of <4; 4-9, >9 mg/m^3 inhalable dust (38). Exposure to higher concentrations may have influenced the effects on lung function decline, although probably not strongly (35). The risk of rapid decline of FEV_1 occur at exposure levels >4 mg/m^3 inhalable dust (53). A significant decrease in FEV_1 and FVC was seen in grain workers over the work day and over the work week at exposure levels of 0.9 ±0.7 mg/m^3 respirable dust and 5.7 ±10.9 mg/m^3 non-respirable dust (19).

Conclusions

Effects on the respiratory system as symptoms and decline in lung function are the critical effects. No NOAEL (no observed adverse effect level) or LOAEL (lowest observed adverse effect level) can be assessed, but effects have been found at concentrations below 4 mg/m^3 inhalable dust.

Table 4. Effects at various concentrations of grain dust.

Exposure (mg/m^3)	Effects	Ref.
10.3-253 total dust	Itchy eyes, nasal congestion, wheezing, cough, mucus expectorations, chronic bronchitis.	24
0.9 ±0.7 respirable dust 5.7 ±10.9 non-respirable dust	Bronchial obstruction and constriction.	19
<5 (3.3 ±7.0) total dust	Higher prevalence of cough, expectorations, shortness of breath, wheezing, eye irritation, nasal congestion.	27
5.4-6.3 total dust	Irritation of eyes and nose, coughing, shortness of breath.	13
>5 total dust	Rapid decline in FEV_1, in excess of 100 ml/year.	31
0-4 inhalable dust	"Chronic bronchitis" and "wheezing ever" more common than in unexposed.	38
dose-response pattern (0-4, 4-9, >9)	Decrease in FEV_1, PEF, MMEF, FEF_{75}.	
<4 total dust	More phlegm production and lower FVC than controls.	35
4-9 total dust	Phlegm, dyspnea, decline in FEV_1, FVC, MMF.	
>4 inhalable dust	Rapid decline of FEV_1.	53

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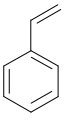
Consensus Report for Styrene

April 1, 2009

This document is an update of the Consensus Report published in 1980 (143) and the revision thereof published in 1991 (102). It incorporates information from a monograph published in 2002 by the International Agency for Research on Cancer (IARC) (71) and risk estimates made by the Harvard Center for Risk Analysis (31). Other sources of information include reports and evaluations by the National Toxicology Program (101, 122) and the Agency for Toxic Substances and Disease Registry (5) in the USA, the Health Council of the Netherlands (64), and the Health and Safety Executive in the U.K. (68). Literature searches have been made and updated regularly, and a final search of Medline was made in November of 2008. Abbreviations used in the text are written out in Appendix 1 at the end of the report.

Chemical and physical data

CAS No.:	100-42-5
Synonyms:	ethenylbenzene, phenylethene, phenylethylene, vinylbenzene
Formula:	C_8H_8
Structure:	
Mol weight:	104.15
Boiling point:	145 °C
Melting point:	- 31 °C
Vapor pressure:	867 Pa (25 °C)
Saturation concentration:	8567 ppm (25 °C)
Density:	0.91 g/cm^3
Distribution coefficient: Log $P_{octanol/water}$	2.95
Conversion factors:	1 mg/m^3 = 0.23 ppm (25 °C) 1 ppm = 4.26 mg/m^3 (25 °C)

Styrene is a clear, viscous liquid with a penetrating odor. It polymerizes readily at room temperature in the presence of oxygen, and oxidizes on exposure to air and light (2). It does not dissolve easily in water (310 mg/l at 25 °C) (113), but is soluble in acetone, diethylether and ethanol (35).

The reported half time for styrene in air is 7.3 hours (108). The odor threshold for styrene has been reported to be 0.32 ppm (2), although an odor threshold of 50 – 80 ppb is reported by the Institute of Environmental Medicine at the Karolinska Institute (72) and 16 ppb is given in the WHO air quality guidelines (158).

Occurrence, use

Low levels of styrene occur naturally in some foods (e.g. cinnamon).

Styrene is commercially available as a 99.6 – 99.9% pure monomer containing at most 10 ppm of the polymerized product.

Styrene is a component of several types of resins used in manufacturing a wide range of products. The six most common resins are polystyrene (building material, packaging material), styrene-butadiene rubber (tires and other vehicle components), unsaturated polyester resins in fiberglass-reinforced plastic (boats, storage tanks, bathtubs/shower stalls), styrene-butadiene latex (backings for floor coverings and paper) acrylonitrile-butadiene styrene (household and office equipment), and styrene acrylonitrile (household products, battery casings).

About 190,000 tons of styrene were used in Sweden in 2005 (Product register, Swedish Chemicals Agency, http://apps.kemi.se/flodessok/floden/flodessok.cfm?lang=eng).

The Swedish National Board of Occupational Safety and Health (now the Swedish Work Environment Authority) mapped styrene use in Sweden in 1997 (3). It ranged from boat hulls and silos to small products such as hoods and bathroom sinks. Production methods were spraying, hand laminating and casting.

Exposure

Background exposure

Low environmental levels of styrene (about 1 ppb) are quite common, since styrene is used industrially on a large scale and is also formed in combustion, and therefore occurs in vehicle exhaust and cigarette smoke (31). The maximum exposure from styrene in food and drink kept in polystyrene containers has been estimated to be about 9 µg/day (about 3 ppb, or about 3 µg/kg foodstuff) (95). Styrene exposure via food – from the food itself and from preparation and storage in styrene containers – has been estimated to be less than 0.2 µg/kg b.w./day (calculated for a body weight of 70 kg) (31).

Occupational exposure

Air levels in workplaces are generally below 10 ppm, but can be higher around work with fiberglass-reinforced plastic (31). Most epidemiological and exposure

studies have been focused on the handling of unsaturated polyester resins during production of fiberglass-reinforced plastic: boats, house trailers, vats, bathtubs etc. During the lamination process, when the resin is applied by hand or sprayed into open molds, styrene concentrations in the surrounding air can far exceed the Swedish exposure limit of 20 ppm (8-hour time-weighted average). A study made in 1996–1999 and covering 328 employees in various fiberglass production facilities in the northeastern USA reported a median value of 9.14 ppm for all examined subjects (135). The highest median value, 45.1 ppm (range 6.74 – 117 ppm) was reported for 48 persons who made recreational vehicles. In these work-places the levels of styrene oxide were about 500 times lower than the styrene levels (135).

In a survey of styrene exposure in ester plastics industries in Sweden, 15% of measurements exceeded 20 ppm, the occupational exposure limit for styrene (3).

Uptake, biotransformation, excretion

With occupational exposure to styrene, the primary path of uptake is via the lungs. Pulmonary uptake of styrene has been calculated in several inhalation studies (70, 154), and reported uptakes in these studies have ranged from 59 to 89%. Concentrations of styrene in blood and exhaled air drop rapidly within the first hour after exposure stops. Styrene accumulates in fat tissue and is excreted in urine, most of it as mandelic acid (MA) and phenylglyoxylic acid (PGA) (71, 154). The half time depends largely on the duration and intensity of exposure. Uptake and disposition of inhaled ^{13}C styrene were measured in 4 male volunteers who were exposed to 50 ppm styrene during light exercise (50 W): it was found that the half times for excretion in urine were on average 3.1 hours for MA and 9.2 hours for PGA (78). The half time for styrene in fat tissue has been calculated to be about 2 to 4 days (48).

The measured rate of skin uptake varies widely from study to study (79). In one study with human skin, the rate of uptake was reported to be 0.06 mg/cm^2/hour (12). Skin uptake was measured by determining styrene in exhaled air and styrene metabolites in urine of subjects who had held one hand in liquid styrene for 15 or 30 minutes. This study was considered to be the best available study of skin uptake by humans (79).

Applying the ECETOC criteria for skin notation (i.e. exposure of 2000 cm^2 skin for 1 hour) (45) to a skin uptake rate of 0.06 mg/cm^2/hour (12) yields a dose of 120 mg via skin uptake. Assuming inhalation of 10 m^3 air in 8 hours and 50% uptake, this is equivalent to 26% of the dose absorbed via inhalation at the present Swedish exposure limit of 90 mg/m^3. Skin exposure to liquid styrene can thus result in a significant addition to the body burden.

Skin uptake of styrene in vapor form is reported to be low: 0.0009 mg/cm^2/hour with whole-body exposure to 600 ppm (128). In another study, skin uptake with exposure to 300 – 735 ppm styrene vapor was calculated to be 5% of inhalation uptake (160).

In a study that compared urine excretion of styrene metabolites in four workers who made fiberglass boat hulls when they wore protective clothing and/or respiratory protection or used no protective equipment, it was concluded that percutaneous absorption of styrene vapor is not a significant pathway of exposure (97). The average air concentration of styrene during the monitored tasks was 49 ppm.

Styrene is transformed via several different cytochrome P450 enzymes to R- and S- stereoenantiomers of styrene 7,8-oxide (SO) and to the ring-oxidized metabolite 4-vinylphenol (50, 71, 105, 117, 166) (Figure 1). In humans, more than 95% of absorbed styrene is initially transformed to SO (78), and metabolites of 4-vinylphenol are estimated to make up about 1% of the total amount of metabolites (105). Results from studies with human liver indicate that CYP2E1 is the cytochrome P450 isoform responsible for metabolism of styrene at low substrate concentrations (84, 116, 157). Two cytochrome P450 enzymes, CYP2A13 and CYP2F1, which are expressed in respiratory passages, may be important in metabolism of styrene in the lungs (27, 50, 117). In addition to the cytochrome P450-dependent oxidation of styrene, oxyhemoglobin has been shown to catalyze the formation of SO from styrene in red blood cells (151).

Styrene metabolism is saturated in humans at air concentrations of about 100 – 200 ppm (99). A maximum metabolic capacity of 0.92 µmol/kg b.w./minute, with an individual variation of 50% (Coefficient of Variation = 0.5) was derived from physiologically based toxicokinetic modeling (parameters based on a total of 24 volunteers in 3 studies who were exposed to 50 – 386 ppm during rest or light exercise) (81).

Figure 1 shows the main metabolic pathways in humans. SO is effectively hydrolyzed via microsomal epoxide hydrolase to styrene glycol, which after several further metabolic steps results in MA and PGA, which are excreted in urine. After 8 hours of inhalation exposure (100 ppm styrene), 57% of the absorbed dose is recovered in urine as MA and 33% as PGA. The relative proportion of MA and PGA (the MA/PGA quotient) in urine at the end of the exposure was 3 ± 1, and 14 hours later 0.8 ± 0.3 (59). A small amount of the SO (about 1% in humans) may be conjugated to glutathione (GSH) and form R- and S-diastereoisomeric forms of the specific mercapturic acids N-acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine (M1) and N-acetyl-S-(2-phenyl-2-hydroxyethyl)-L-cysteine (M2), which are also excreted in urine (30). The variation in urine levels of the metabolites M1 and M2 has been ascribed to differences in the ability to conjugate to glutathione via the glutathione transferase enzymes GSTM1 and GSTT1 (63). The decisive role of the GSTM1 genotype in excretion of M1 and M2 was clearly shown in urine analyses of two workers who had been accidentally exposed to styrene (104). Levels of M1 and M2 were about five times lower in one of the workers, who lacked GSTM1 activity. The metabolism of 4-vinylphenol is understood in less detail, but it has been suggested that further

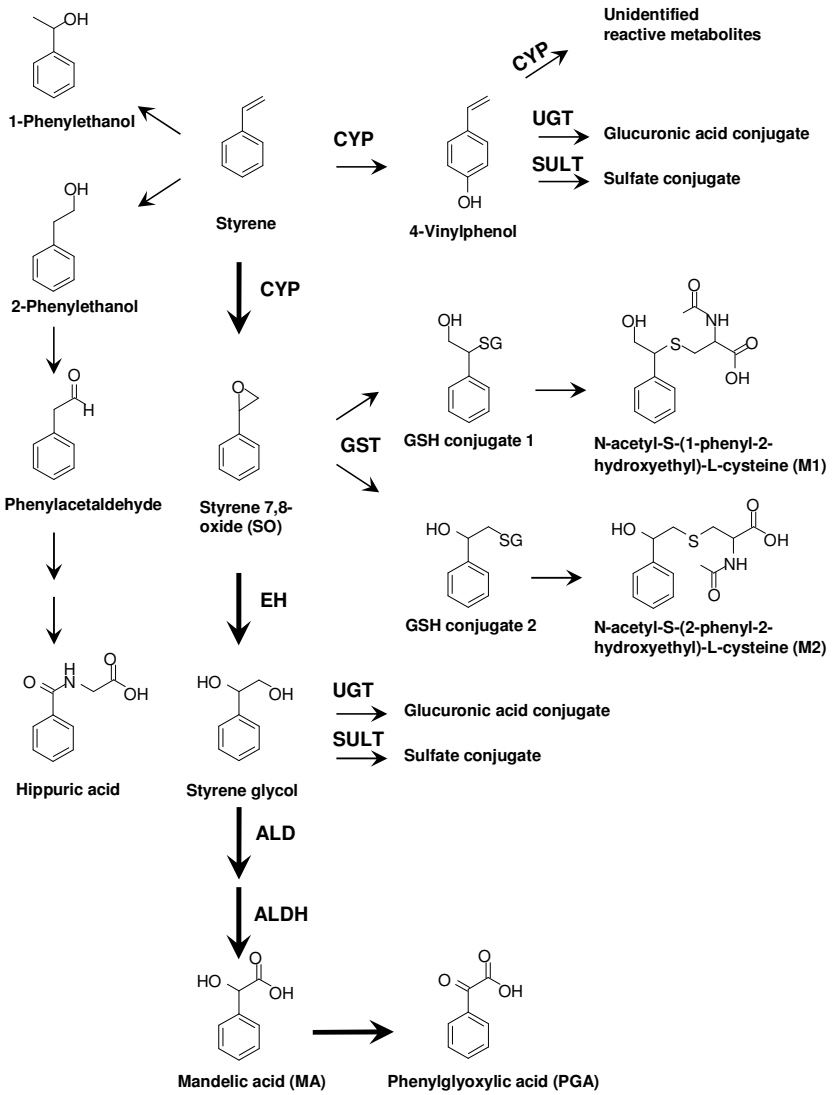


Figure 1. The main pathways for metabolic breakdown of styrene. The pathways accounting for the greater amounts in humans are indicated by the thicker arrows. ALD: alcohol dehydrogenase, ALDH: aldehyde dehydrogenase, CYP: cytochrome P450, EH: epoxide hydrolase, GSH: glutathione, GST: glutathione transferase, SULT: sulfotransferase, UGT: glucuronosyl transferase.

metabolism via the enzymes CYP2E1 and CYP2F gives rise to toxic metabolites (22, 23, 167). Conjugation to glucuronic acid and sulfate is followed by excretion of conjugates of 4-vinylphenol in urine.

Styrene metabolism in humans shows wide individual variation, and seems to be induced not only by repeated exposure to styrene but also by exposure to ethanol (98, 99, 120, 121). Induction of styrene metabolism by ethanol and by caloric restriction has also been shown in studies with rats (30, 132). Styrene induces several different cytochrome P450 enzymes in exposed rats (65). mRNA expression of the enzyme CYP2E1 in lymphocytes, which is a biomarker for CYP2E1 activity, shows positive correlations to excretion of the mercapturic acids M1 and M2 (in individuals with the GSTM1-positive genotype) and to urine levels of MA (63). Overall, CYP2E1 stands out as the enzyme most important to both toxicity and metabolism of styrene in humans. The greatest difference between rats and mice with regard to styrene metabolism seems to be that the amounts of metabolites formed via ring oxidation to 4-vinylphenol and via phenylacetaldehyde are 4 to 10 times greater in mice. In people, metabolism via SO and further hydrolysis to MA and PGA seems to be by far the most dominant path, and metabolism via phenylacetaldehyde accounts for much less than it does in rats and mice (38).

A number of recently published studies focus on metabolism of styrene to cytotoxic metabolites in lungs, and results of several of them indicate that local metabolism in Clara cells in terminal bronchioles plays a central role (see below under “Inter-species differences in sensitivity”).

Biological exposure monitoring

Biological exposure monitoring has attractive advantages. It provides an integrated dose measure over time, reflects differences in workload as well as exposure via both inhalation and skin uptake, and shows the effects of protective equipment. Several different markers/methods for biological exposure monitoring of styrene have been described, including styrene in blood or urine, metabolites in urine (hippuric acid, MA, PGA, or PGA+MA), styrene in exhaled air, styrene oxide or styrene glycol in blood, mercapturic acids in urine (M1 and M2, see Figure 1), and adducts of hemoglobin and albumin in blood (for a review of methods see Reference 1). The latter methods, using protein adducts, yield integrated exposure estimates for longer periods (weeks or months) (1, 145). Glucuronic acid- and sulfate-conjugated metabolites of 4-vinylphenol have also been proposed as biomarkers for styrene exposure (105). Of these markers, MA, PGA and MA+PGA in urine are the ones most widely used and best validated (1).

The American Conference of Governmental Industrial Hygienists (ACGIH) bases its biological exposure limit for styrene on the sum of the metabolites MA and PGA in urine, and recommends a biological exposure index of 400 mg MA+PGA/g creatinine in samples taken within one hour after a workshift, which is reported to correspond to occupational exposure to an air concentration of about 20 ppm. This urine level reflects exposure for the day, and to some extent for the previous day. MA and PGA are not specific indicators of styrene exposure,

however, since other substances (e.g. ethylbenzene) also produce the same metabolites and/or interact (e.g. ethanol, toluene) with the metabolism of styrene. It is therefore recommended that, in order to confirm specific styrene exposure, blood styrene be measured also, and a (semiquantitative) biological exposure index of 0.2 mg/l is recommended for styrene in blood taken directly after a workshift (1).

In this report, where biological markers are used to estimate occupational exposure levels for styrene, the following correlations have been applied: 400 mg MA+PGA or 300 mg MA/g creatinine in urine samples taken directly after a workshift corresponds to an 8-hour exposure to an air concentration of 20 ppm styrene (1).

Toxic effects

Effects on the central nervous system and sense organs

The review below is limited to studies made since 1990, when the previous Consensus Report on Styrene was published, and takes up only those studies performed in accordance with accepted standards and containing exposure information useful in determining occupational exposure limits.

Acute effects

Table 1 presents the two reports found in the literature search that deal with acute central nervous system (CNS) effects on experimentally exposed people (133, 140). Both of these studies were made with several exposure schedules and exposure levels, but neither of them could demonstrate any effects of styrene at the tested levels (up to 6 hours at 50 ppm).

Effects of occupational exposure

Effects on the sense organs and CNS documented in studies of occupationally exposed workers are presented in Tables 2, 3 and 4.

Effects on results of psychological performance tests

Table 2 summarizes the reported results of psychological performance tests, and presents information from three original studies (77, 149, 159) and a report containing a meta-analysis (8). All the studies in Table 2 show effects of styrene exposure at levels near the present Swedish occupational exposure limit of 20 ppm (90 mg/m^3).

In the meta-analysis, the authors review several studies in which the reaction time of workers exposed to styrene was tested. The review covers four studies of choice reaction time (tests in which the subject has to choose the appropriate response to several simple signals) and three in which simple reaction time was tested. The authors report significant correlations between choice reaction time and a measure of cumulative styrene exposure. Their calculations indicate that eight years of exposure to a styrene concentration of 20 ppm yields a 6.5% increase in reaction time (8).

Table 1. Acute effects registered in human subjects experimentally exposed to styrene by inhalation.

Exposure level	Exposure time	Number of subjects	Tests used	Effects	Ref.
14 ppm (varying: 0.5–40 ppm)	4 hours	24	Simple and choice reaction time Attention	No effects.	133
20 ppm	3 hours	16	Simple and choice reaction time Attention	No effects.	133
5, 25, 50 ppm (constant) + 25, 50 ppm (varying)	6 hours	24	Color vision Contrast sensitivity Odor threshold Simple and complex reaction time Memory Coordination	No effects.	133, 140

Table 2. Occupational exposure to styrene and effects on results of psychological performance tests.

Exposure level	Years	Type of study	Effects	Ref.
20 ppm [a]	8	Meta-analysis [b]	6.5% increase in choice reaction time.	8
21.9 ppm [c] (range 0–181 ppm)	8.3 ± 7.9	Cross-sectional: 41 exposed 45 controls	Prolonged reaction time. Elevated vibration thresholds.	149
30 ppm [d] (20% of measurements > 50 ppm)	5 ± 4.5	Cross-sectional: 30 exposed 30 controls	Prolonged reaction time.	77
36 ppm [c]	< 10 [e]	Cross-sectional: 90 exposed 64 controls	Prolonged reaction time. Worse results on coding test.	159

[a] 20 ppm is the exposure level that has significant effects after 8 years of exposure, as calculated by linear regression.
[b] Of the studies included, 2 (77, 149) are reviewed in this document.
[c] Average value.
[d] Calculated by the authors from metabolites in urine. Average value was 722 mg MA+ PGA/g creatinine (95% CI: 610 – 833).
[e] Time employed is given in the original paper as number of hours doing lamination work.

In a French study, test results of 30 boat builders exposed to styrene levels generally below 50 ppm were compared with those of 30 employees at the same company who were not exposed to styrene. The groups were matched for ethnic background, sex, age, and intellectual and sociocultural levels. Noise levels for

the two groups were about the same. All measurements were made on Mondays, and the tests were given both before and after the workshift. Three tests of attention and memory were given, and on the test days MA and PGA in urine were measured and air levels of styrene were measured individually with passive sampling and stationary monitors at each workstation. The average air level of styrene was 22.7 ppm (range 4 – 55 ppm). Levels of MA+PGA were 37.6 (range 0 – 165) mg/g creatinine in the morning before exposure, and 574.8 (range 90 – 2180) mg/g creatinine after the workshift. There was a relatively good correlation between styrene in air and metabolites in urine: $r = 0.73$ ($p < 0.001$). Metabolites were also measured in post-shift urine samples for ten months, and the average value was 722 mg/g creatinine (95% CI: 610 – 833). According to the authors, this level corresponds to an average exposure of about 30 ppm, although levels above 50 ppm were indicated by 20% of the samples. The exposed subjects did worse than controls on all three tests, both before and after exposure, but when they were divided into three exposure groups no clear dose-effect relationship could be seen. Since the differences between exposed subjects and controls were independent of acute exposure, the authors interpret their results as clear indication of long-term effects of styrene exposure (77).

In a cross-sectional study of a retrospective cohort at a boat building plant in Belgium, a group of workers previously exposed to styrene (n = 90) and a group with low-level current exposure (n = 27) were tested and their results were compared with those of a control group (n = 64). The previously exposed workers were tested three to four years after their exposures had stopped. An earlier study (published only in Dutch) reports that 4 – 9% of time-weighted averages exceeded 50 ppm and 4% of short-term values were above 100 ppm. Company records contained information that allowed computation of the exact number of exposed hours for each worker, as well as the exposure level at the corresponding points in time. Average exposure during 1982 – 1989 was 35 ppm for the workers who were still employed and 37 ppm for those who were no longer exposed. At the time of the study the average exposure level for the employed group was 9.6 (SD ± 5.6, range 2.7 – 28.2) ppm. The authors refer in their conclusions to an exposure level of 155 mg/m^3, which is about 36 ppm. Both of the exposed groups did worse than controls on the “Symbol digit” (a coding test) and number memory tests. The results of a coordination test were worse only for the previously exposed group. These differences could be seen even after several confounding variables were included in the statistical model. Only the number of years doing exposed work correlated with performance: i.e. intensity of exposure was not related to performance (159).

In a study from Taiwan, published in 1996, 41 exposed workers and 45 controls from six companies producing fiberglass-reinforced plastic were examined. Of the original group of 177 persons, 25 with less than 6 years of education were excluded because they were not able to take the tests. A further 26 persons refused to take the tests, and 40 persons were excluded for medical reasons. On the day of the study 88 exposure measurements were made with personal monitors and 22

with stationary monitors. The subjects were divided into two groups based on these readings, and those with the lowest exposures (0 – 6.4 ppm) were put in the control group. Time-weighted average exposure for the exposed group was 21.9 ppm, with a median value of 8.6 ppm (range 0 –181), and number of years in exposed work was 8.3 ± 7.9. The subjects filled out questionnaires and were given psychological performance tests, and vibration thresholds and temperature thresholds were also measured to examine function of the peripheral nervous system. Significant differences between the groups could be seen in a test of attention and in vibration thresholds in both hands and feet (149).

Effects on color vision

The color vision test used in most of the studies reviewed here is “Lanthony D15”. This test consists of 15 color samples in unsaturated pastel colors, which are to be ordered according to color. The test was originally developed to differentiate between different types of color vision defects, but because of its sensitivity it has been widely used in occupational toxicology examinations for the past 20 years. The results are usually reported in the form of a Color Confusion Index (CCI), which reflects the severity of errors made in sorting. The mechanism behind the observed effects on color vision is not known – that is, it is not known whether they result from an effect on the nervous system or from some effect on the eye itself. The extent to which the effects are reversible is also unknown, but some reversibility is suggested in some studies.

Twelve reports regarding effects on color vision in styrene-exposed workers are reviewed below. Nine of these are original reports (20, 46, 49, 52, 53, 57, 73, 74, 87, 147), one is a statistical calculation of a critical exposure level based on results of two studies (21), and two are meta-analyses (8, 125). The relevant studies are summarized in Table 3.

A study made in Japan includes 87 exposed workers and 87 controls, all of whom were men. Stationary monitors showed exposure levels in the range 7 – 36 ppm. The exposed subjects were divided into three groups on the basis of MA in urine: < 0.1 g/l, 0.1 – 0.2 g/l, and > 0.2 g/l. According to the authors, these levels correspond to styrene exposures of up to 8 ppm, 8 – 16 ppm and above 16 ppm. The average MA values for the three groups indicated exposures of about 4, 10, and 46 ppm respectively. Both the medium- and high-exposure groups had poorer color vision than their matched controls. There was also a dose-effect relationship ($r = 0.38$; $p < 0.001$) between MA in urine and performance on the color test (87).

In 2002 this research group published a further color vision study. The subjects were boat builders exposed to styrene. Exposure levels were calculated on the basis of metabolites (MA+PGA) in urine, and the subjects were divided into high (above 10 ppm) and low (up to 10 ppm) exposure groups of 29 persons each and matched for age to a mixed control group of 29 persons. The results for the two exposed groups and the controls were analyzed. Both of the exposed groups had significantly worse results on the color test than the control group (57).

Table 3. Effects on color vision from occupational exposure to styrene.

Exposure level (ppm)	Years of exposure	Type of study	Effects	Ref.
< 10 [a] > 10 [a]	–	Cross-sectional: 29 high exposure 29 low exposure 29 controls	Significant difference from controls for both exposure groups. Weak dose-effect correlation.	57
< 8 [a] 8 – 16 [a] > 16 [a]	6	Cross-sectional: 21 low exposure 24 med. exposure 42 high exposure 87 controls	Medium- and high-exposure groups showed effects when compared to controls. Dose-effect correlation.	87
16	–	Cross-sectional: 36 exposed 36 controls	Significant difference. Dose-effect correlation.	53
16.2 (range 1-129)	7	Cross-sectional: 41 exposed 41 controls	Poorer color vision in the exposed group.	52
20 [b, c]	8	Meta-analysis of 6 different studies [d]: Calculation of level for significant effect.	2.3% deterioration in color vision, equivalent to 1.7 years of aging.	8
9 [c] (range 1-13) 23 [e] (range 14-30)	12.9 17.8	Cross-sectional: 55 low exposure 53 high exposure	Significant difference between groups. Weak dose-effect correlation (p=0.52).	73, 74
24.3	6.5	Cross-sectional: 60 exposed 60 controls	A significantly higher number of persons in the exposed group had mistakes on a color vision test.	49
< 30 [a] > 30 [a]	7	Cross-sectional: 40 low exposure 17 high exposure 57 controls	Significant difference between high-exposure group and controls.	46

[a] Calculated from metabolites in urine (see section on “Biological exposure monitoring”).
[b] Statistical calculation of the level at which significant effects appear.
[c] Calculated from the different dose measures used in the different studies.
[d] Of the six studies reviewed in the meta-analysis, five are reviewed in this report.
[e] Calculated average exposure for entire working life.

A Swedish study of styrene-exposed workers, based on data collected during 1998 – 1999, was published in 2005. At the time of the study, exposure for the 108 participating workers ranged from 1 to 20 ppm. Results of an individually calculated index for average career exposure (Lifetime Weighted Average Exposure, LWAE) ranged from 1 to 30 ppm. The workers were divided into two groups on the basis of this index: average exposures for the two groups were 9 (range 1 – 13) and 23 (range 14 – 30) ppm. Comparison of results on the color test showed a significant difference between the two groups, and a weak dose-effect

relationship with the historical exposure data could be seen. There was, however, no correlation between color perception and current exposure (73, 74).

One of the earliest studies reporting effects on color perception due to exposure to relatively low levels of styrene was published in 1991 by scientists in Italy. In a study of color perception in a group of 41 exposed workers and their matched controls, they report a dose-related effect of about 7 years of exposure to an average 16.2 ppm (geometric mean; range 1 – 129 ppm) (52).

Two years later the same research group published a study of color perception in 36 exposed workers and their matched controls. This study was published as a part of a larger study on levels of styrene metabolites in urine. Here as well, exposures were about 16 ppm (geometric mean), and color perception was poorer in the exposed group. A dose-effect correlation could also be seen (53).

Another early study showing effects of low-level styrene exposure on color perception was published in 1992. This report covers 60 exposed workers at a shipyard, who were compared with a control group matched for age, social status and ethnic background. On the day of the test the air level of styrene was 24.3 ppm, and the post-shift level of MA+PGA in urine was 287 mg/g creatinine. Although there was little difference between the groups in total number of errors on the color perception test, a significantly larger proportion of the exposed group made mistakes suggesting that color vision was affected. There were also differences in performance on a coordination test (49).

Color vision was tested in a group of 57 styrene-exposed workers and their matched controls. The average exposure level for the exposed workers, as shown by stationary monitors, was 18.5 (range 6.6 – 36.4) ppm. The exposed workers performed more poorly than controls. When they were divided into two groups on the basis of MA level in urine – up to 30 ppm for the first group, and over 30 ppm for the second – and the two groups were compared with age-matched controls, there was a group difference only for the group exposed to over 30 ppm (46).

In 2001 a research group in Germany published what they called an “intervention study” of a small group of 22 styrene-exposed laminators and 11 controls. Color perception was tested Monday morning and Thursday afternoon of the same week, on a total of 6 occasions: before and immediately after a 4-week vacation, and again on the same schedule about 10 months later, after exposure levels had been reduced. Styrene exposure was determined by measurements of MA+PGA in urine samples taken in conjunction with the Thursday afternoon tests. Color vision had improved appreciably after the 4-week vacation. When the subjects were examined 10 months later, color vision had improved further on the corresponding test occasions and test scores had returned to “normal range”. The authors concluded that styrene has a dose-dependent effect on color perception (147). They assert that the effect is reversible, which their data suggest, but neglect to point out that even at the lowered exposure there was a tendency for color vision to deteriorate during the workweek. They also assert that “The current BAT value (600 mg MA+PGA per gram creatinine) protects employees from this non-adverse effect.”

Visual acuity, color perception and contrast sensitivity were tested in a study of 128 styrene-exposed boat builders at three companies in Canada. Stationary monitors indicated an average exposure level of 48 ppm (SD = 61, first quartile 5 ppm, median 10 ppm, and third quartile 89 ppm). MA in urine was also determined. After exclusion of 47 persons (most of them for medical reasons), the remaining 81 persons, 79 men and 2 women, were included in the statistical analyses. Their average age was 29 (SD = 8), and they had been employed for an average of 5 years (SD = 4). The results of the analyses showed that both color perception and contrast sensitivity are negatively affected by styrene exposure, and a dose-effect relationship could be seen (20).

Data from the studies described above by Gobba *et al.* (52) and Campagna *et al.* (20) were used by a group of scientists from Canada and Italy (21) in an attempt to statistically calculate a lowest observed effect level (LOEL). The basis for their analysis consisted of 118 workers, 67 from Canada and 51 from Italy. The mathematical model they used indicates a LOEL of 4 ppm, with an upper limit of 26 ppm for the 95% confidence interval (21). This model has not won general acceptance within risk assessment, however, and the authors themselves seem a bit uncertain of their interpretation of the results.

In a meta-analysis of results from six different studies (21, 46, 52, 54, 57, 87) a group of American scientists calculated the effects that can be expected from eight years of occupational exposure to 20 ppm styrene (the present exposure limit). The meta-analysis yielded significant results: the effect of 8 years of exposure to 20 ppm styrene is equivalent to the deterioration of color vision associated with an age increase of 1.7 years (8).

One more report will be reviewed here, although it contains no new findings. It also is a meta-analysis, and includes seven different groups in six original studies (46, 52, 53, 86, 87, 147). It was made by a research group in Dortmund. Although each of these studies reports significant findings, the authors of the meta-analysis contend that the meta-analysis did not yield significant results. The reason for this lack of significance is the large variability in effect sizes over the different studies. The authors discuss several possible reasons for this absence of significance, but arrive at no definite conclusion (125).

Effects on hearing and balance

A number of studies throwing light on the connection between styrene exposure and effects on hearing and balance have been published in recent years. One problem in assessing these studies is that the noise exposure of the studied groups is often not reported, or is at best incompletely described. Another is that historical data are often lacking. Below is a brief review of results from studies showing correlations between low-level exposure to styrene and effects on hearing and balance (Table 4).

Table 4. Effects on hearing and balance observed in cross-sectional studies of workers occupationally exposed to styrene. (S = styrene exposure; N = noise exposure; C = controls)

Exposure level (ppm styrene)	Job tenure (years)	Number of subjects	Effects	Ref.
7.5 [a]	7.1 (SD=6.2)	32 S 60 C	Hearing thresholds for most tested frequencies were significantly higher in exposed subjects than in controls.	106
14.5 ± 12.2 [b]		290 S 223 C	Odds quotient for hearing loss with styrene exposure was 3.9 (95% CI: 2.4 – 6.2) after correction for age, sex, and current and previous noise exposure. There was a significant positive linear correlation between average career exposure to styrene and elevated hearing thresholds at 6 and 8 kHz.	141 142
18 [c] 3.8 [d] 9.1 [e]	17 (1–39) S 12 (1–35) N 18 (2–38) C	65 S 78 N 81 C	Persons exposed to styrene but not high noise levels had significantly higher hearing thresholds at some frequencies than either controls or persons exposed to noise only.	112
15.6 [f] 3.2 [g] 9.1 [c]		154 S and S+N 159 N and C	In the final multiple logistic regression analysis, only current noise level, age and MA level in urine significantly affected hearing thresholds. An increase of 152 mg MA/g creatinine in urine was associated with an odds ratio of 2.44 for hearing loss (95% CI: 1.01–5.89).	
25 ± 28 S 5 ± 10 C		88 S 88 C	There were significant effects on several variables in the balance test, even after relatively brief exposures.	146
37 (range 3–98)	7.6 (2–25)	20 S 10 C	Effects on balance test. No effects noted in audiometry or auditory brainstem response.	19

[a] Calculated from values of 149 ± 80 mg MA+PGA/g creatinine in urine.
[b] Calculated career average (range 0.8 – 72.5 ppm).
[c] Calculated career average.
[d] Measured air levels (range 0.1 – 22.5 ppm).
[e] Calculated from an average value of 136.8 mg MA/g creatinine in urine (range from below the detection limit to 30 ppm). The value is an underestimate because of the dilution effect (see text).
[f] Average career exposures to styrene and styrene+noise were calculated by the Criteria Group.
[g] Measured air level. Averages for styrene and styrene+noise were calculated by the Criteria Group.

A Polish research group (141, 142) examined effects on hearing from occupational exposure to solvents (styrene alone or in combination with toluene) in the plastics and fiberglass boat industries. The studied group consisted of 290 persons (250 exposed to styrene only) with average career exposures of 14.5 ± 12.2 ppm (range 0.8 – 72.5 ppm) for styrene and 82.1 ± 4.3 dB (range 71.3 – 93.0 dB) for noise. The reference group consisted of 223 office and factory workers not exposed to solvents. Career exposure to noise was on average 73.2 ± 5.3 dB for the office workers (n = 157) and 89.2 ± 3.1 dB for the factory workers (n = 66). Audiograms for 63.1% of the exposed group and 41.7% of the reference group showed deviations, and the multiple logistic regression analysis yielded an odds ratio of 3.9 (95% CI: 2.4 – 6.2) for hearing loss related to styrene exposure, after adjustment for age, sex, and current and previous noise exposure. There was also a significant positive linear correlation between average career exposure to styrene and elevated hearing thresholds at 6 and 8 kHz.

A research group in Finland published a study of 252 styrene-exposed workers who made fiberglass boats (146). They tested balance, measured air levels of styrene for 148 workers with passive samplers, and determined MA and PGA in urine. Two groups, one consisting of 88 "laminators" and the other of 88 workers with lower exposures, were matched for age. The calculated average air level of styrene was 108 mg/m^3 (SD = 119), or 25 ppm, for the high-exposure group, and 21 mg/m^3 (SD = 42), or 5 ppm, for the low-exposure group. The group with higher exposure showed poorer performance for several parameters on the balance test.

In a cross-sectional study of personnel at 11 Swedish companies, Morata *et al.* (112) examined hearing loss in persons exposed to low levels of styrene and/or high noise levels. Four groups were compared: one group exposed to styrene (n = 65), one exposed to styrene and high noise levels (n = 89), one exposed to high noise levels (n = 78), and a control group (n = 81) exposed to neither styrene nor high noise levels. For the two groups exposed to noise, the average noise levels were respectively 85 dB and 89 dB (range 75 – 116 dB). The average noise level was 82 dB (range 75 – 84 dB) for the styrene-exposed group and 77 dB (range 69 – 86 dB) for the controls. Styrene exposure was measured with personal monitors for a day, and was 3.8 ppm (range 0.05 – 22.5 ppm) for the styrene-exposed group and 2.8 ppm (range 0.01 – 11.7 ppm) for the group exposed to both styrene and noise. On the day the styrene levels were monitored, 24-hour urine samples were taken starting at the beginning of the shift. Average MA levels were 137 mg/g creatinine (range from below the detection limit to 441) in the styrene-exposed group, and 137 mg/g creatinine (range from below the detection limit to 456) in the group exposed to both styrene and noise. The correlation between MA levels in urine and air levels of styrene was significant but low ($p < 0.001$, $r = 0.27$). Career exposure to styrene was also estimated, using information in company records. Calculated career exposure was 306 ppm x years (average exposure time 17 years) for the group exposed to styrene, and 208 ppm x years (average exposure time 15 years) for the group exposed to both styrene and noise. Persons exposed to

styrene (with or without high noise levels) had significantly higher hearing thresholds at 2, 3, 4 and 6 kHz than both controls and those exposed to noise alone. Of several variables included in the final multiple logistic regression analysis, only current noise level, age and MA level in urine significantly affected hearing thresholds. An increase of 152 mg MA/g creatinine in urine carried an odds ratio of 2.44 (95% CI: 1.01 – 5.89) for hearing loss, i.e. an exposure increase of about 10 ppm more than doubles the risk of hearing loss. No statistically significant interactions between styrene and noise (other than purely additive) could be shown (112). Calculating the air levels of styrene from MA levels in urine (see section on "Biological exposure monitoring"; a post-shift level of 300 mg MA/g creatinine corresponds to an air level of 20 ppm styrene (1)) yields an air level of 9.1 ppm (range from below the detection limit to about 30 ppm) for the two groups exposed to styrene. This is an underestimate due to a dilution effect, however, since the urine samples were collected over 24 hours.

A more thorough assessment of data from the study described above was published by the same authors in 2006 (80). They report analyses of more detailed hearing tests, and conclude that the effects on hearing are probably not in the ear alone, but also in the CNS.

A small group of 20 exposed workers was studied by Calabrese *et al.* (19), who tested hearing and balance and measured electrical activity in the brain. Exposure was measured with passive sampling and also estimated from styrene metabolites in urine. Average exposure was 37 ppm (SD = 29), with a range of 3 – 98 ppm. Urine samples were collected on Friday morning before the day's exposure. MA+PGA was on average 348 mg/g creatinine (SD = 197). Data for the exposed workers were compared with reference data for the tests, and no effects could be seen on audiometry or auditory brainstem responses (ABR), although several parameters on the balance test were affected.

A conference proceedings published in 2007, probably without peer review, contains a study of a small group of styrene-exposed workers at a large Italian boatyard (106). The average noise level at their workplace was 73 dB(A), which normally does not damage hearing. These workers were compared with a control group. The two groups were also checked for noise exposure away from work, family history of hearing loss, previous hearing problems, and use of medications with known ototoxic effects. Noise at the workplace was measured. Styrene exposure was determined by post-shift measurements of MA+PGA in urine, and hearing thresholds were measured at several frequencies (0.5 – 1 – 2 – 3 – 4 – 6 – 8 kHz). Measured levels of MA+PGA in urine corresponded to an exposure of 7.5 ppm styrene in air. The 20 exposed men and 12 exposed women were matched for age and sex to a control group of 60 persons. The matching yielded 20 men and 4 women in each group, and the statistical analysis showed that the exposed subjects had somewhat less acute hearing at all tested frequencies except 8 kHz in the right ear. The hearing loss was at most 25 dB.

Hearing thresholds in a group of laminators (n = 127) and other workers (n = 127) who made fiberglass-reinforced boats were tested on two occasions: during a

workweek and in conjunction with time off work. On the latter occasion they had been exposure-free for 7 to 23 days. The subjects were divided into three groups (low, medium, high exposure) on the basis of metabolites in urine. Average metabolite levels for the three groups were 51, 229 and 970 mg MA+PGA/g creatinine (equivalent to 2.5, 11.5 and 48.5 ppm styrene, see section on "Biological exposure monitoring". Effects were seen only at exposure levels of 30 – 50 ppm and after many years of exposure (148).

Ischemic heart disease (IHD)

In a mortality study, 40,688 persons who worked with reinforced plastic in Denmark, Finland, Italy, Norway, Great Britain and Sweden were followed and causes of death were compared with rates for the national populations. Mortality due to circulatory disease was lower than projected: the Standardized Mortality Ratio (SMR) in the exposed groups ranged from 0.91 to 0.97 (88).

Mortality due to ischemic heart disease was also lower among workers who develop and manufacture styrene-based products than among the national population in the USA: SMR = 0.81 (95% CI: 0.71 – 0.92) (15).

These results are in conformity with the assumption that, in general, the working population is healthier than the national population as a whole, which contains many people unable to work.

A cohort of 5,204 persons who produced plastic boats in two companies in the USA between 1959 and 1978 was followed until 1998. At one of the companies the average exposure was 42.5 ppm in the fiberglass department and at the other 71.7 ppm in the laminating department. These were the high-exposure departments. Neither of these departments showed a significant elevation in risk for IHD. However, mortality due to respiratory diseases was higher among these workers than in the populations of the state of Washington (SMR 2.07; 95% CI: 1.24 – 3.23) and the USA as a whole (SMR 1.92; 95% CI: 1.15 – 3.00) (129).

A total of 15,826 people worked for at least six months with production of reinforced styrene plastic in 30 factories in the USA during the years 1948 – 1977. Styrene exposure ranged from 1 to 200 ppm. The cohort was followed until 1989 and causes of death were compared with national mortality rates. A total of 1,628 people in the cohort died. There was no significant elevation in mortality due to IHD (SMR 1.04; 95% CI: 0.94 – 1.14), although there was an elevation in mortality due to IHD for persons employed less than one year (SMR 1.29; $p < 0.05$) (168). Persons exposed or employed for less than a year usually have a higher mortality rate than persons with longer/steadier employment, but this elevation in mortality is generally due more to mental illnesses and external causes than to IHD (13).

In one study, 498 cases of IHD were compared with a random selection of 997 persons employed during the 1943 – 1984 period in two styrene-butadiene rubber plants in the USA. Both styrene and butadiene exposures were estimated, and ranked from 0 to 10 on an exposure matrix containing 579 job assignments. Mortality due to acute IHD was elevated among those who had been exposed

to styrene during the preceding two years (Relative Risk 4.99; 95% CI: 1.7 – 23.34). There was no significant correlation to chronic IHD, but for acute IHD there was a dose-response correlation, with an elevation in risk when styrene exposure during the previous two years exceeded 0.2 ppm. For exposures above 0.3 ppm the risk elevation was 4 (Relative Risk 4.3; 95% CI: 1.6 – 11.8) (108).

A later study examines mortality due to IHD in a total of 16,579 men who worked in styrene-butadiene rubber plants in the USA and Canada. This study also includes the two production plants of the Matanoski and Tao study (108). No significant correlation between styrene exposure during the previous two years and mortality due to acute IHD could be observed in this study. There was, however, a correlation between average lifetime styrene exposure and chronic IHD for persons below the age of 55 (44).

There is thus one study that raises suspicions of a connection between IHD and exposure to styrene (108), and a larger study with similar design that was unable to confirm such a connection (44). One possible explanation for this discrepancy may be that the study reporting an elevated risk (108) included all employees, whereas the other study (44) included only persons who had been employed for at least a year.

Effects on respiratory passages

Styrene at air levels of about 20 ppm is reported to cause irritation of eyes, throat and respiratory passages (70). Welp *et al.* investigated the question of whether styrene causes non-malignant lung diseases in the cohort of 34,560 men and 6,128 women who participated in the European lung cancer study reported by Kogevinas *et al.* (88), see below under "Carcinogenicity, Human data". It was found that pneumonia as cause of death had a significant correlation (p for trend 0.01) to intensity of styrene exposure (average exposure level) but not to cumulative exposure. Mortality due to bronchitis, emphysema and asthma showed no correlation to styrene exposure (156).

Toxic effects on respiratory passages have also been documented in experiments with rats and mice (Table 5). Mice are more sensitive than rats: hyperplasias in nasal epithelium and lungs have been reported with chronic exposure to 20 ppm for mice and 50 ppm for rats (36, 37, 38). Cell proliferation in terminal bronchioles has also been observed in mice after oral doses of 100 mg/kg/day for 5 days (58). Reduced levels of Clara cell protein (CC16) and morphological damage in the terminal bronchioles of mice exposed to styrene indicate that Clara cells are the target cells for cytotoxicity in the lungs (24, 25, 37, 38, 51, 62, 83). Styrene's toxic effects on respiratory passages are assumed to be due primarily to its oxidation to SO and to the less common ring-oxidized metabolite 4-vinylphenol, which is more toxic than SO in lungs of mice (22 – 26, 39, 83, 167).

Table 5. Toxic effects observed in the respiratory passages of laboratory animals exposed to styrene by inhalation.

Exposure (ppm)	Exposure schedule	Species	Effects	Ref.
20	6 hrs/day, 5 days/week, 104 wks (males) 98 wks (females)	CD-1 mice	Degeneration and atrophy in nasal mucosa, and in females also a significant increase in bronchial adenomas.	37
40	6 hrs/day, 3 days	CD-1 mice	Cell proliferation in lungs.	58
40	6 hours	CD-1 mice (females)	Elevated levels of ALP and LDH and reduced level of CC16 in pulmonary lavage; reduced serum levels of CC16.	51
160	6 hrs/day, 5 days	CD-1 mice (females)	Destruction of Clara cells in lungs.	51
50	6 hrs/day, 5 days/week, 104 weeks	Sprague-Dawley rats	Low-level but dose-related histopathological changes in nasal mucosa of all animals killed week 52 and week 104.	36

Other toxic effects

Hepatotoxicity with exposure to styrene during work with fiberglass-reinforced plastic has been reported in two independent studies (16). Significant and exposure-dependent elevations in bilirubin (direct) and elevated transaminase activity (ALAT and ASAT) are described. The first study had 47 exposed subjects and 14 controls, average exposure was 21.8 ppm and average length of employment was 3.8 years. The second study had 21 exposed subjects and 26 controls, average exposure was 24.1 ppm, and average length of employment was 5.5 years.

Results of animal experiments indicate that styrene causes severe but reversible oxidative stress in the liver (26, 150). A single intraperitoneal dose of 600 mg/kg given to mice produced a reversible drop in reduced glutathione (GSH) levels and a compensatory activation of γ-glutamylcysteine synthetase (26, 150). Histological changes and initally lowered levels of reduced glutathione in the liver were also reported in rats exposed to 300 ppm styrene 6 hours/day, 5 days/week for 11 weeks (153).

Oxidative damage, including effects on viability, permeability, and levels of reduced glutathione and antioxidant enzymes, as well as DNA strand breaks (comet assay), have been reported with *in vitro* exposure of human skin to styrene levels of 100 ppm or higher (34).

There are a few reported cases of allergic contact eczema caused by styrene (32, 138, 139). Styrene was classified as a weak (138) or mild/moderate (unclear which) (134) contact allergen in the Guinea Pig Maximization Test. Since styrene is a widely used substance and skin contact must be quite common, it can be concluded that styrene rarely causes contact allergy.

Genotoxicity

In vitro data

The ability of styrene to damage genetic material has been widely tested in several different systems. In its most recent (2002) genotoxicity assessment of styrene, the IARC reports that SO (both with and without metabolic activation) causes mutations in Ames' tests with *Salmonella*. Styrene, on the other hand, was reported to yield mostly negative results in Ames' tests for mutagenicity. Some positive results were reported after metabolic activation (71).

Styrene does not react with DNA, but SO forms covalent bonds with DNA and proteins. SO has been shown in several *in vitro* studies to form N^2 and O^6 adducts of guanine in DNA in exposed mammalian cells (71, 166).

Promutagenic damage in the form of DNA strand breaks and fragmentation has been observed in human lymphocytes exposed *in vitro* to relatively high concentrations of styrene. This concurs with observations of adducts of DNA and proteins (71). Results of five recently published *in vitro* studies reporting DNA damage to human lymphocytes exposed to high doses of styrene and SO are presented in Table 6.

It has been demonstrated that styrene is metabolized in blood to genotoxic metabolites (6). Chinese hamster cells (V79 cells), cultivated on slides, were exposed to styrene (2.4 and 4.8 mM) for 4 hours in a rat liver perfusion system containing red blood cells (hemoglobin) from cows. They were placed directly after the liver so that the perfusate washed over the V79 cells. A notable

Table 6. Genetic damage observed in human cells exposed *in vitro* to styrene or styrene oxide.[a]

System	Dose	Effect	Result	Ref.
Lymphocytes	Styrene 0.5 and 1.5 mM	Sister chromatid exchanges.	Positive (1.5 mM)	11
Lymphocytes	Styrene 5 and 10 mM	DNA fragmentation (comet assay).	Positive (5 mM)	94
Lymphocytes	Styrene oxide 10, 20, 50, 100 and 200 µM	DNA fragmentation (comet assay). Micronuclei. Sister chromatid exchanges.	Positive (50 µM) Positive (50 µM) Positive (50 µM)	91
Lymphocytes	Styrene oxide 10, 25, 50, 75 and 100 µM	DNA fragmentation (comet assay).	Positive (25 µM)	18
Lymphocytes	Styrene oxide 50 and 200 µM	DNA fragmentation (comet assay). Micronuclei.	Positive (50 µM) Positive (50 µM)	93
Human lymphoblastoid cell line GSTM1(-)	Styrene oxide 1–2 mM	Gene mutations (HPRT) [b]	Positive (1 mM)	137

[a] These studies were not included in the IARC evaluation published in 2002.
[b] HPRT = hypoxanthine phosphoribosyltransferase

observation was that the presence of red blood cells was necessary for the appearance of mutations. The authors eliminated SO as the DNA-reactive metabolite formed because SO is very rapidly metabolized by the perfused liver and also because it caused no mutations in V79 cells exposed to 2.08 mM SO for up to four hours.

Animal data

In some, but not all, animal experiments, exposure to high concentrations of styrene has been reported to increase frequencies of chromomsome aberrations, sister chromatid exchanges and micronuclei. In the IARC evaluation published in 2002, these results are not considered to be convincing evidence of cytogenetic damage in experimentally exposed animals (71). Some studies published since the IARC evaluation are summarized in Table 7.

Mice were exposed to 750 or 1500 mg/m^3 (176 or 352 ppm) styrene 6 hours/day for 1, 3, 7 or 21 days: a dose-dependent increase of DNA adducts in lungs was observed, and there was a significant increase in the number of micronuclei in bone marrow on day 7, but not on day 21 (163). The number of micronuclei in bone marrow on day 7 had a significant correlation to DNA strand breaks on day 7, but not at other times. The study was repeated with addition of a further sampling occasion on day 14 (47). The repeated study yielded no evidence of an exposure-dependent increase of micronuclei in bone marrow.

Recently published genotoxicity assessments of styrene have consistently concluded that styrene has a weak damaging effect on chromosomes in experimental animals (31, 101, 166).

Table 7. Types of genetic damage observed in laboratory animals exposed to styrene by inhalation. [a]

Exposure	System	Effect	Result	Ref.
125 or 250 ppm, 6 hours	CD-1 mice, liver	Unscheduled DNA synthesis.	Negative	29
0, 40 or 160 ppm, 6 hours/day, 1, 5 or 20 days	CD-1 mice, lungs	8-OH-deoxyguanosine.	Negative	51
0, 160 or 500 ppm, 6 hours/day, 1, 5 or 20 days	Crl:CD rats, lungs	8-OH-deoxyguanosine.	Negative	51
352 ppm, 7 or 21 days	NMRI mice, bone marrow	SO-adenine and SO-guanine. DNA strand breaks (comet assay). Micronuclei.	Positive Weakly positive Weakly positive [b]	163
352 ppm, 1 to 21 days	NMRI mice, bone marrow	Micronuclei.	Negative	47

[a] These studies were not included in the IARC evaluation published in 2002.
[b] Significant after 7 days but not after 21 days.

Human data

N^3 and O^6 adducts of SO to guanine have been identified in lymphocytes of styrene-exposed workers (161, 162, 164, 166). Hemoglobin adducts and O^6 and N^2 deoxyguanosine DNA adducts correlate to external exposure measurements and to biomarkers in urine (164).

Using an alkalic elution method, Walles *et al.* showed in 1993 that the number of DNA single-strand breaks in white blood cells from 17 styrene-exposed workers in a Swedish plastics factory was significantly correlated to the amount of styrene in air and to the amount of MA in urine (155). The levels in workplace air were below the Swedish exposure limit of 20 ppm, averaging 7 ppm (range 0.4 – 20 ppm). Blood and urine samples were taken before and after a workshift and on the following morning. Only the after-shift measurements showed significant correlations between DNA strand breaks and intensity of exposure, measured as styrene in blood, styrene in urine and MA in urine. The authors concluded that most of the DNA damage was rapidly repaired.

Use of the comet method has increased sensitivity in detection of single-strand breaks in DNA, and in studies of styrene-exposed workers made in the past few years strand breaks have been found in DNA from both lymphocytes and sperm (18, 55, 92, 109, 110). Significant correlations have been found between DNA strand breaks and internal dose measures such as DNA adducts or hemoglobin adducts in people occupationally exposed to styrene (164). Along with adducts in N-terminal valine in hemoglobin, which is the most sensitive marker of styrene exposure, the presence of DNA adducts and strand breaks indicates electrophilic activity.

Further, lymphocytes from workers exposed to styrene have an elevated ability to repair DNA damage: this has been observed *in vitro* in cells treated with 0.1 mM SO (55) or with x-ray radiation (5 gray) (165). These results can be regarded as evidence that ongoing exposure to styrene induces DNA repair enzymes.

The IARC, on reviewing about 30 studies of occupational exposure to styrene in various industries, found some evidence of a quantitative correlation between styrene exposure and frequency of chromosome aberrations, but not frequencies of sister chromatid exchanges or micronuclei. Elevated occurrence of chromosome aberrations was reported in 12 of 25 studies, sister chromatid exchanges in 6 of 16, and micronuclei in 3 of 14 studies. Similarly, an expert panel at the Harvard Center for Risk Analysis judged that there is evidence of exposure-related chromosome aberrations in styrene-exposed persons, but less evidence for the occurrence of sister chromatid exchanges and micronuclei (31). Since the IARC review, a further 11 studies of genotoxic effects/DNA damage in workers exposed to styrene have been published. The results are summarized in Table 8, which also contains information from the above-mentioned 1993 study documenting strand breaks in DNA at very low styrene levels (155).

Three of the studies (55, 111, 165) are very well made, reported in detail, and based on groups of 44 to 95 exposed subjects. They show positive correlations

between micronuclei in lymphocytes and styrene metabolites in urine at low exposure levels.

A study of 86 Czech laminators, which included 16 unexposed workers and 26 external referents, reports statistically significant correlations between the frequency of binuclear lymphocytes with micronuclei and the level of styrene in air ($p<0.001$) and in blood ($p<0.001$) (165). The average air level for all exposed subjects was 19.1 ± 13.2 ppm (mean ± SD).

A Belgian study of 44 men making fiberglass-laminated products and 44 matched male referents from two factories producing electric cables reports significant differences between exposed subjects (9.5 ± 9.6 ppm) and referents with regard to numbers of micronuclei in mononuclear lymphocytes ($p<0.001$), binuclear lymphocytes ($p = 0.02$), and nasal lavage fluid ($p = 0.04$) (55).

For the 95 Italian laminators (76 men and 19 women) examined in one of the studies by Migliore *et al.* (111), post-shift levels of styrene metabolites in urine were 300 ± 338.2 mg MA+PGA/g creatinine. Air levels of styrene (8.7 ± 0.9 ppm) and urine levels of the glutathione conjugates M1 and M2 and the ring-oxidized metabolite 4-vinylphenol were determined for 45 subjects, and showed strongly significant correlations. This study also showed clear correlations between micronuclei in lymphocytes and the number of chromosome aberrations. Both chromosome aberrations and micronuclei showed significant correlations to MA+PGA and to 4-vinylphenol. In a figure in the article that charts the correlations between level of 4-vinylphenol and number of binuclear micronuclei per 10^3 cells (Figure 2a) and frequency of centromere-positive micronuclei (Figure 2b), it appeared that two subjects with extremely high exposures might account for the significance of the correlations between these effect markers and levels of 4-vinylphenol. In the process of compiling this document, Lucia Migliore was contacted: she supplied the raw data and could confirm that the correlations remained significant if these two subjects were removed from the calculations. It was observed that individuals with the GSTT1-null genotype had significantly higher levels of micronuclei among the styrene-exposed but not among the unexposed. Since GSTT1 is expressed in erythrocytes but not at all in lymphocytes, this finding may indicate that metabolism of styrene to electrophilic metabolites (which can be conjugated and detoxified by GSTT1) in erythrocytes may play a role in the occurrence of DNA damage in lymphocytes.

In a work published in 2003 (164), Vodicka *et al.* present a compilation of several studies they had published in 1993 – 2001. They review data on chromosome aberrations in lymphocytes from 75 styrene-exposed subjects that show strong correlations between the level of chromosome aberrations and styrene levels in air ($R^2 = 0.212$; $p = 0.0001$) and in blood ($R^2 = 0.24$; $p = 0.001$).

Table 8. Genetic effects observed in subjects occupationally exposed to styrene [a]. The studies are listed chronologically.

Material	Exposure	Effect	Result	Ref.
Lymphocytes 17 exposed	Laminators. 7.0 ppm styrene (average, n=16; range 0.4–20); 70 mg MA/g creatinine (average, n=17; range nd–261) post-shift.	Single strand breaks (alkalic elution).	Positive	155
Lymphocytes 14 exposed 70 controls	Laminators. 3 monitoring occasions: 313.2 ± 90.9, 324.8 ± 71.4, 352.8 ± 153.1 mg MA/g creatinine (average ± SE). Air levels of styrene estimated by the authors: 16.8 ± 5.9 ppm, 17.5 ± 4.6 ppm, 19.3 ± 10 ppm (average ± SE) post-shift.	DNA fragmentation (comet). Micronuclei. Sister chromatid exchanges.	Positive Positive Positive	92
Lymphocytes 26 exposed 26 controls	Laminators. 240 mg MA+PGA/g creatinine (median, range 98–382) post-shift.	Single strand breaks (DNA unwinding).	Positive	136
Sperm 46 exposed 27 controls	Laminators. 28% exposed >20 ppm styrene; 173.6 mg MA/g creatinine (median, range 5.8–1428.7) post-shift.	DNA fragmentation (comet).	Positive	109
Sperm 18 exposed 13 controls	Laminators. 295.8 mg MA/g creatinine (average, range 20.8–947.8) post-shift. At least 2 years of exposure.	Numerical changes in chromosomes.	Negative	115
Lymphocytes	Combined studies.	SO-DNA adducts, n=30. Hb adducts, n=76. Gene mutations (HPRT), n=72. DNA fragmentation (comet), n=58. Chromosome aberrations, n=79.	Positive Positive Positive [b] Positive Positive	164
Lymphocytes (genotyped) 48 exposed 14 controls	Laminators and polymer production workers. 36.8 ± 0.7 ppm styrene (geometric mean ± GSD, range 1.2–115.7); 205.5 ± 2.4 mg MA+PGA/g creatinine (geometric mean ± GSD, range 31.6–1412.5) post-shift.	DNA fragmentation (comet).	Positive	18
Lymphocytes (genotyped) 86 exposed 42 controls	Laminators. 19.1 ± 13.2 ppm styrene (average ± SD).	DNA fragmentation (comet). Micronuclei. Chromosome aberrations. Elevated DNA repair ability.	Negative Positive [c] Negative Positive	165

Table 8. Continued.

Material	Exposure	Effect	Result	Ref.
Lymphocytes (genotyped) 44 exposed 44 controls	Laminators. 201.6 ± 148.3 mg MA/g creatinine (average ± SD, range nd–618.2) post-shift. Air levels of styrene estimated by the authors: 9.5 ± 9.6 ppm (average ± SD, range 0–36.6).	Hb adducts. DNA fragmentation (comet). Micronuclei in lymphocytes. Micronuclei in nasal lavage.	Positive [d] Positive [e] Positive Positive	55
Lymphocytes (genotyped) 28 exposed 28 controls	Laminators. 27 ± 5 ppm styrene (average ± SE, range 2 – 91); 401 ± 73 mg MA+PGA/g creatinine (average ± SE, range 47–1490) morning after shift.	Sister chromatid exchanges. Micronuclei.	Positive Negative	144
Lymphocytes (genotyped) 95 exposed 98 controls	Laminators. 8.7 ± 0.9 ppm styrene (average ± SD, range 0.47–125.6), n=45; 300 ± 338.2 mg MA+PGA/g creatinine (average ± SD, range 10.2–1856.0), n=95; post-shift.	Micronuclei. Chromosome aberrations.	Positive Positive	111
Lymphocytes Sperm 42 exposed 25 controls	Laminators. 630 ± 35 mg MA+PGA/g creatinine.	Micronuclei in lymphocytes. DNA fragmenation (comet) in sperm.	Positive [f] Positive [f]	110

[a] Not included in the 2002 IARC evaluation.
[b] Significant positive correlation to air levels of styrene but not to MA in urine. Significant correlation between HPRT (hypoxanthine phosphoribosyltransferase) mutations and cumulative exposure time.
[c] Significant positive correlations to all exposure markers. Average for exposed subjects significantly different from workplace controls but not significantly different from average for external controls.
[d] Significant positive correlation to urine levels when blood samples were taken but not to average levels in urine samples taken weekly for 3 months.
[e] Significant positive correlation to Hb adducts but not significantly higher than unexposed subjects.
[f] Significant correlation between DNA fragmentation in sperm and micronuclei in lymphocytes for 67 subjects (both exposed and controls) but no correlation to metabolites in urine.

Variations in metabolism and repair probably explain quite a bit of the individual differences in sensitivity to styrene, and probably also lie behind the difficulty in establishing dose-response relationships based on exposure levels in air. In many of the studies presented in Table 8, attempts were also made to identify sensitive groups by genotyping for biotransformation enzymes or DNA-repair enzymes. Because the groups were small, however, most of the presented results, which identify bearers of certain polymorphic variants of some biotransformation enzymes and DNA repair enzymes, are very tenuous and need to be verified. Conclusions on genetically determined sensitivity to styrene are therefore not reviewed or further commented here. A study of micronuclei in 644 individuals (pooled data from studies of 343 occupationally exposed and 301 unexposed subjects), published in 2006, reports that the occurrence of micronuclei is

significantly higher in women and also that it increases with age (85). It is likely that in some of the early studies a correlation between styrene exposure and occurrence of micronuclei went undetected because of age and sex differences between exposed groups and controls.

To sum up, there are data from several studies of styrene-exposed subjects that show exposure-related elevations in DNA strand breaks and other cytogenetic changes. These findings must be placed in the context of our general knowledge about what cytogenetic changes mean in terms of cancer risk. In follow-ups of individuals who have been tested for cytogenetic changes in several contexts (not only in studies of occupational exposure) it has been observed that elevated levels of chromosome aberrations in lymphocytes are an indicator of increased cancer risk (60, 61). Further, a recent study in the the Human MicroNucleus project has shown that micronuclei in peripheral lymphocytes of healthy persons constitute a biomarker predictive of elevated cancer risk (14). The study reports higher total cancer incidences in groups with elevated numbers of micronuclei. These groups also had significantly lower survival. Bladder cancer and renal cancer, as well as stomach and intestinal cancer, were significantly correlated to high frequencies of micronuclei.

Using the rad-equivalence method, the ability of SO to form micronuclei was compared to that of gamma radiation (56). Comparison of the doses of SO and gamma radiation that yielded similar amounts of micronuclei in human lymphocytes exposed *in vitro*, along with knowledge of the carcinogenic potency of styrene oxide, allowed calculation of a lifetime risk of developing fatal lymphatic hematopoietic cancer: 0.17 (range 0.037 – 5) per 1000 persons occupationally exposed to 20 ppm styrene for 40 years.

Carcinogenicity

The IARC has assessed the carcinogenicity of styrene and classified it as “possibly carcinogenic to humans” (Class 2B) (70, 71).

Animal data

Eight chronic toxicity/cancer studies have been made with rats. Exposures were via inhalation, gavage or drinking water (Table 9). In its 2002 evaluation (71), the IARC stated that “there was no reliable evidence for an increase in tumour incidence in rats”.

Female rats were exposed by inhalation to 0, 25, 50, 100, 200 or 300 ppm styrene for 52 weeks, and malignant mammary tumors were found in 6/60 (10%), 6/30 (20%), 4/30 (13%), 9/30 (30%), 12/30 (40%) and 9/30 (30%) respectively. Total incidences of mammary tumors at these exposure levels were respectively 34/60 (57%), 24/30 (80%), 21/30 (70%), 23/30 (77%), 24/30 (80%) and 25/30 (83%) (33).

Table 9. Styrene carcinogenicity studies with rats.

Exposure	Strain	Result	Ref.
Gavage: 500 mg/kg b.w., 1 day/week, 120 weeks	BDIV	Negative.	126
Inhalation: 600 or 1000 ppm, 6 hours/day, 5 days/week, 18.3 months (males), 20.7 months (females)	Sprague-Dawley	Positive for mammary tumors (adenocarcinomas) in low-dose group.	31*
Gavage: 500 mg/kg b.w., 5 days/week, 103 weeks; 1000 or 2000 mg/kg b.w., 5 days/week, 78 weeks	F344/N	Negative.	118
Gavage: 30% β-nitrostyrene, 70% styrene, 175, 350 or 700 mg/kg b.w., 3 days/week, 79 weeks	F344/N	Negative.	119
In drinking water: 125 or 250 ppm, 104 weeks	Sprague-Dawley	Negative.	7
Gavage: 50 or 250 mg/kg b.w., 4 or 5 days/week, 52 weeks	Sprague-Dawley	Negative.	33
Inhalation: 25, 50, 100, 200 or 300 ppm, 4 hours/day, 5 days/week, 52 weeks	Sprague-Dawley	Positive for both total and malignant mammary tumors.	33
Inhalation: 50, 200, 500 or 1000 ppm, 6 hours/day, 5 days/week, 104 weeks	Sprague-Dawley	Positive in the two highest dose groups for benign tumors in interstitial cells in testes.	36

* Jersey *et al.*, Dow Chemicals, 1978; cited in Reference 31.

Regarding the positive results for mammary tumors reported in the inhalation study by Conti *et al.* (33), the assessments of the IARC (71) and HCRA (31) both point out that exposure time was short, that the reporting was incomplete, and that the frequency of spontaneous tumors was high; both mention also that in the well-reported study by Cruzan *et al.* (36) a dose-dependent reduction of mammary tumors was seen in females. In this study rats were exposed to 0, 50, 200, 500 or 1000 ppm styrene for 104 weeks, and mammary adenocarcinomas were observed in 20/61 (33%), 13/60 (22%), 9/60 (15%), 5/60 (8%) and 2/60 (3%); fibroadenomas, including epithelial hyperplasias, were observed in 27/61 (44%), 22/60 (37%), 18/60 (30%), 21/60 (35%) and 19/60 (32%) respectively.

Three of five studies with mice have provided evidence that styrene causes lung tumors, but no evidence of tumors of other types (Table 10). In one study, groups of 50 male and 50 female CD-1 mice were exposed to 0, 20, 40, 80 or 160 ppm styrene for 98 weeks (females) or 104 weeks (males). Elevated occurrences of benign lung tumors were reported in mice exposed to styrene levels as low as 20 – 40 ppm, and malignant tumors at 160 ppm. Bronchoalveolar adenomas were found in 15/50 (30%), 21/50 (42%), 35/50 (70%), 30/50 (60%) and 33/50 (66%) males, and in 6/50 (12%), 16/50 (32%), 16/50 (32%), 11/50 (22%) and 24/50

Table 10. Styrene carcinogenicity studies with mice.

Exposure	Strain	Result	Ref.
Gavage: 1350 mg/kg b.w., 1 day/week, lifelong	O_{20}	Positive for lung tumors in both sexes.	126
Gavage: 300 mg/kg b.w., 1 day/week, lifelong	C57B1	Negative.	126
Gavage: 150 or 300 mg/kg b.w., 5 days/week, 78 weeks	$B6C3F_1$	Significantly higher occurrence of broncho-alveolar adenomas and carcinomas in males in the high-dose group.	118
Gavage: 30% β-nitrostyrene, 70% styrene, 200 or 400 mg/kg b.w., 3 days/week, 78 weeks	$B6C3F_1$	Negative.	119
Inhalation: 20, 40, 80 or 160 ppm, 6 hours/day, 5 days/week, 98 weeks (females), 104 weeks (males)	CD-1	Positive for late lung tumors. Elevated occurrence of lung adenomas at all exposure levels. Significant increase of lung carcinomas only in females at 160 ppm.	37

(48%) females exposed to 0, 20, 40, 80 and 160 ppm respectively. Malignant bronchoalveolar carcinomas were reported in 0/50 (0%), 0/50 (0%), 2/50 (4%), 0/50 (0%) and 7/50 (14%) females at these exposure levels. There was no increase of malignant lung tumors in the males (37).

Administration of SO by gavage has been shown to increase the incidence of tumors in the forestomachs of both rats (33, 96, 127) and mice (96). In its evaluation, the IARC states that there is "sufficient evidence" that SO causes tumors in experimentally exposed animals (70).

In summary, the assessments made by the IARC (71), HCRA (31) and ATSDR (5) all conclude that styrene causes lung tumors in mice. There is no reliable evidence that styrene causes tumors in rats.

Human data

This section does not contain detailed reviews of the numerous published studies of potentially carcinogenic effects on humans, but rather provides a survey of the principal findings that are described in more detail in the risk assessments made by the IARC (71), HCRA (31), EU (68), and ATSDR (5). A few recent follow-ups of earlier studies are also reviewed. The IARC assessed 13 retrospective cohort studies, five of which drew their material from industries producing fiberglass-reinforced plastic. The others were from styrene monomer and styrene polymer production and from production of styrene-butadiene rubber (71). As previously mentioned, the highest exposure levels occur in production of fiberglass-reinforced plastic, where also mixed exposures are less common. A problem here is that short-term employment is widespread in this industry, and epidemiological studies are consequently quite difficult.

Studies of styrene-exposed mice have indicated that there may be a risk of lung cancer associated with styrene exposure (37). Most of the epidemiological studies, however, have reported no elevation in risk for lung cancer in any of the three different types of industry where styrene occurs (15, 66, 88, 123, 131). In a cohort of 15,826 men and women employed in production of fiberglass-reinforced plastic, however, there were 162 cases of lung cancer vs. 115.2 expected (SMR 1.41; 95% CI: 1.20 - 1.64) (168). The elevation in risk was seen only at low cumulative exposures and in persons with brief employment.

An early study of workers making styrene-based products reports a significant increase of lymphatic leukemia (124). Follow-up studies, however, have yielded equivocal results for lymphatic and hematopoietic cancers (15, 88, 89, 103, 107, 169). A multinational European study of nearly 41,000 employees working with fiberglass-reinforced plastic at 660 different companies, which included eight subcohorts from Denmark, Finland, Italy, Norway, Sweden and the UK, reported no increase in either overall mortality or mortality due to malignant neoplastic diseases (88). Mortality from neoplastic diseases in lymphatic and hematopoietic tissues increased with time elapsed from initial exposure and with increasing average exposure, but not with cumulative exposure.

No increase in mortality due to leukemia or lymphoma is reported in the study by Ruder *et al.* (see above under "Ischemic heart disease"), which is an update of a cohort study of 5204 persons who made fiberglass boats in two companies in the USA between 1959 and 1978 and who were followed until 1998 (129). Elevated mortalities due to cancers of the esophagus (n = 12, SMR 2.30; 95% CI: 1.19 – 4.02) and prostate (n = 24, SMR 1.71; 95% CI: 1.09 – 2.54) were reported, and among 2062 highly exposed workers there was also an elevated incidence of urinary tract cancer (n = 6, SMR 3.44; 95% CI: 1.26 – 7.50).

Other studies of laminators have also reported elevated mortalities due to various forms of cancer, including cancers of the esophagus (88, 169), pancreas (88), urinary tract (88, 123, 168), male sex organs (123, 168) and female sex organs (168). Most of these increases were small, not statistically proven, and not seen in groups with the highest exposures, and have therefore been considered to be unrelated to styrene exposure (31).

There are thus a number of epidemiological studies in which possible increases in mortality due to various forms of cancer have been observed among people exposed to styrene. The IARC pointed out in its assessment of the epidemiological data that the risk increases were generally low and statistically uncertain and were often based on analyses of sub-groups. The findings were rather weak, and the possibility that they were due to chance, bias or simultaneous exposure to other substances could not be ruled out.

Inter-species differences in sensitivity

The local metabolism in the lungs – particularly in Clara cells – that leads to formation of toxic metabolites has been put forth as an explanation for the differences between mice and rats in sensitivity to lung tumors. The hypothesis

that the greater sensitivity of mice may be explained by higher levels of SO in terminal bronchioles still has some support, but the observation that, at the same exposure levels, blood levels of styrene and SO are the same in mice and rats argues against a causal role for SO (37).

Metabolic transformation of styrene to SO in lung tissue from mice, rats and humans has been compared in several studies (summarized in Reference 31). These studies have shown that cytochrome P450-dependent transformation to SO is less effective in human lung than in rat lung, and much less effective than in mouse lung.

Both physiologically based pharmacokinetic models and measurements of SO in *in situ* ventilated lungs show higher local concentrations of SO in mouse lung, but this difference does not seem large enough to provide a complete explanation for the difference between rats and mice in sensitivity to lung cancer (42, 67). Protection against toxic effects in Clara cells with P450 inhibitors, lower levels of SO in the blood of mice at levels of styrene that cause lung tumors (160 ppm) than in rats at higher levels of styrene that do not cause lung tumors (1000 ppm), as well as lack of SO-DNA adducts in lungs, also argue against the blood level of SO as a major factor in the appearance of lung cancer (37, 38, 83). Current research suggests rather that mice have a greater sensitivity to local formation of reactive styrene metabolites and GSH depletion, which leads to local toxicity and compensatory cell proliferation (42, 130). This greater sensitivity in mice, together with the higher activity of the enzymes CYP2E1 and CYP2F2 and the somewhat higher formation of the more reactive and more toxic R-enantiomer of SO in mouse lung, has been put forward as possible explanation for their greater sensitivity to lung tumors (31, 42, 51, 67, 69, 83). It has also been proposed, on the basis of new data, that 4-vinylphenol and its metabolites are more important than SO in the appearance of lung cancer (38, 39, 167). CYP2F2, the enzyme that has been most strongly associated to formation of 4-vinylphenol, has lower activity in lungs of humans and rats than in lungs of mice (17, 58).

Remarkably high concentrations of styrene and SO are needed to cause DNA strand breaks/genotoxicity under *in vitro* conditions. There also seems to be a considerable difference in sensitivity to chromosome damage, including micronuclei, between occupationally exposed people and the animal species exposed in laboratories. In humans, micronuclei have been identified in lymphocytes at exposure levels of about 20 ppm and lower. In animal experiments, on the other hand, only a few studies have reported elevated levels of micronuclei and then at exposures above 300 ppm. In animal exposures, however, microneuclei in bone marrow have been studied rather than micronuclei in peripheral lymphocytes. This may partially explain why experimental animals seem to be less sensitive.

The well-made genotoxicity studies of occupationally exposed humans made in the past few years should be given greater weight than the earlier studies on which the IARC based its assessments (70, 71). More refined methods of detecting exposure markers and biomarkers for genotoxicity have been used in the newer studies, and clear correlations between genotoxic effects and various exposure

markers have been shown. It has also been clearly shown that gender and age are factors having a large effect on biomarkers for genotoxicity, and the recent studies that include larger numbers of subjects have had better control over these confounding factors.

Effects on reproduction

There are only a few studies of styrene's toxic effects on reproductive function, and most of them do not meet present standards. A Dutch hazard evaluation (64) of human and animal studies of styrene published in 1966 – 1999 found no support for classification and labeling of styrene with regard to effects on fertility, developmental toxicity, or effects during lactation. However, the committee strongly emphasized that several of the assessed human studies give reason to fear toxic effects on reproduction even though it is not clear in these studies whether exposure was to pure styrene or to a mixture of solvents including styrene (64). Studies published since this assessment are reviewed below under *Human data* and *Animal data*.

Human data

From 1560 Danish, Italian and Dutch men who worked or had worked with fiberglass-reinforced plastic were chosen 220 styrene-exposed and 382 unexposed (at time of conception) men who already had at least one child. The men were divided into four groups (high, medium, low and no exposure) on the basis of exposure estimates derived from historical styrene measurements and styrene metabolites in urine. Fertility was estimated by reported time to conception. A not-significant reduction in fertility was seen in the exposed men (fertility ratio 0.79; 95% CI: 0.59 – 1.05), but there was no observed reduction with increasing estimated exposure. The men with the highest exposure had a fertility ratio of 1.09 (95% CI: 0.69 – 1.72). The authors concluded that styrene exposure probably has little effect on male fertility (90).

Luderer *et al.* (100) examined correlations between prolactin levels in serum and occupational exposure to styrene in 259 men and 43 women. The subjects were recruited from 17 companies in the plastics industry. Exposure, styrene in blood and prolactin in serum were measured on 1 to 3 occasions about a year apart. On each sampling day, the subject was interviewed and observed working and the blood samples were taken at the end of the workshift. The exposure measurements, made with personal monitors, covered an entire workshift (8 hours) and were made 1 to 5 times (average 3) during the month the samples were taken (median exposure 9 ppm, range <1 – 142 ppm). Adjustments were made for a number of confounding factors, including gender, age, smoking habits, use of alcohol and other drugs, acetone exposure and use of protective equipment. Styrene in blood was more closely correlated to prolactin levels in serum than to styrene levels in air. Persons with exposures above 20 ppm were more likely (OR = 3.69; 95% CI: 1.39 – 9.97) to have an elevated prolactin level in serum (above

the reference values of 15 ng/ml for men and 20 ng/ml for women) than persons with lower exposure. Modeling was used to calculate prolactin levels as a function of acute, subacute or chronic air or blood levels of styrene with linear multiple regression analysis. The correlation to elevated prolactin levels was significant in the acute exposure model, but not in the sub-chronic or chronic model. According to the authors, their results confirm that styrene exposure elevates prolactin levels in serum and provide support for regarding this as an acute effect (100). This study confirms earlier studies reporting that prolactin levels in serum of both men and women occupationally exposed to styrene were higher than in unexposed controls (4, 9, 10, 114). The clinical relevance of elevated prolactin levels as a response to styrene exposure is not clear.

Cho *et al.* (28) examined the occurrence of oligomenorrhea (defined as a menstrual cycle longer than 35 days, including amenorrhea) during the previous year among women exposed to solvents (benzene, toluene, xylene and styrene) in the petrochemical industry. The women were recruited from a government industrial area in China via their applications for permission to marry or become pregnant. Data were collected for 1408 women. A qualitative occupational hygiene estimate of solvent exposure was made for workplaces and jobs, i.e. whether exposure to benzene, toluene, xylene and/or styrene occurred or not. Averages were reported to be low for benzene: 0.017 ppm (rubber industry) to 0.191 ppm (chemical industry). Average levels of toluene, xylene and styrene were reported to be below 1 ppm. A total of 440 women were judged to have been exposed to one or more solvents, and 276 exposed to styrene (3 to styrene only). Oligomenorrhea was reported on a questionnaire by 8.5% of the unexposed women, 12.7% of women exposed to one or more solvents, and 14.5% of women whose exposure included styrene. After adjustment for factors including age, body mass index (BMI), passive smoking and exposure to other chemicals, the odds ratio was 1.34 (95% CI: 0.90 – 1.99) for exposure to one or more solvents and 1.65 (95% CI: 1.05 – 2.55) for exposure that included styrene. The authors concluded that exposure to solvents is associated with a trend toward greater frequency of oligomenorrhea (28).

Animal data

In a two-generation reproduction study (40) rats (25 animals of each sex per exposure group) were exposed by inhalation to styrene concentrations of 0, 50, 150 or 500 ppm, 6 hours/day for at least 70 days prior to mating (F_0 and F_1 generations), and exposure of the females was continued throughout gestation and from day 5 of lactation. On the first 4 days of the lactation period the mothers were given by gavage 66, 117 or 300 mg styrene/kg b.w./day, divided into 3 equal doses 2 hours apart. Indications of systemic toxicity prior to mating, in the form of lower body weights compared to the control group, were seen in F_0 males and females in the 500 ppm group and in the F_1 generation in the 500 and 150 ppm groups (but not significant for females at 150 ppm). Body weights during gestation were significantly lower only in females in the F_1 generation in the 500 ppm

group. No effects were observed in either the F_0 or F_1 generation on mating and fertility index for males or females, pre-coital interval, spermatogenesis, reproductive organ weights, estrous cycle, length of gestation, litter size and sex ratio, and postnatal survival. Further, the numbers of ovarian follicles and corpora lutea in the F_1 generation in the 500 ppm group were the same as in controls. The authors give a no observed adverse effect level (NOAEL) of 50 ppm for systemic toxicity to the parents and 500 ppm or more for effects on reproductive ability (40). Pups (F_2) born to the F_1 generation in the 150 and 500 ppm groups had lower body weights 21 days after birth (not significant for females at 150 ppm). At the higher exposure the lower body weights persisted (not significant) until 70 days after birth and a slight retardation in physical development was noted. Pups in the high-dose group had poorer swimming ability on day 24 after birth, and grip strength was reduced in both sexes on days 45 and 60 after birth. No effects were seen in the acoustic startle response, learning and memory tests, motor activity, brain morphology and histology, or brain weight. No exposure-related effects were seen in the 50 ppm group, and no indications of teratogenicity were seen in any of the exposed groups. The authors give a NOAEL of 50 ppm for growth and 500 ppm for neurological development in the F_2 generation (40, 41).

Rats (10 – 14 per group) were exposed to 0 (controls), 50 or 300 ppm styrene for 6 hours/day on days 6 – 20 of gestation (82). No significant differences in weight gain were seen in the mothers during the exposure period, although a significant reduction (about 20%) of feed intake was seen in the group exposed to 300 ppm. An extra control group was introduced with a corresponding reduction in feed availability (pair-fed control) and the same feed restriction was made for the group exposed to 50 ppm. No significant effects were observed in the group exposed to 50 ppm when they were compared with the pair-fed controls. In the group exposed to 300 ppm there was a significant increase in neonatal mortality, and 21 days after birth the male pups had lower body weights than pair-fed controls. There were also delays in eye opening, tooth eruption and development of the air righting reflex.

In a study with rats, hormone-disturbing effects of styrene, styrene dimer and styrene trimer were examined *in vivo* and *in vitro* (43). *In vitro* there was no observed binding of any of these substances to receptors for estrogen, androgen or thyroxin (tested up to 10 µM). *In vivo* there was no observed effect on uterus weight in either sexually immature (n = 5) or ovariectomized (n = 5) female rats after subcutaneous injection of up to 200 mg/kg b.w./day for 3 days. Nor was there any anti-androgenic effect seen in testosterone-treated, castrated, sexually immature male rats (n = 5) given up to 200 mg/kg b.w./day by gavage for 7 days. Subcutaneous injections of 20 mg/kg b.w./day given to ovariectomized rats for 3 days had no effect on prolactin levels in blood.

Male rats (10 animals/group) were exposed to 0 (controls), 150, 500 or 1500 ppm styrene 6 hours/day for 5 days. When compared with controls, exposed rats showed no significant effect on prolactin or dopamine levels in serum or on the levels of catecholamines and their metabolites in striatum or hypothalamus in

the brain, at any dose level. No difference was seen if the rats were given a day to recover after the exposure. According to the authors, these negative results suggest that styrene probably has no neuroendocrine effects on humans (75). In male rats (10 animals/group) exposed on the same schedule there was a small but significant increase of luteinizing hormone (LH) in serum from the two highest exposure groups immediately after the exposure (76). Another study (152) reports a small but significant increase of prolactin in plasma of female rats, but not male rats, exposed to 150 ppm styrene (8 animals/group) 8 hours/day, 5 days/week for 2 weeks. The female rats had about twice as much styrene in their blood as the males.

Dose-effect/dose-response relationships

Several studies (8, 77, 149) report effects on psychological performance tests after less than 10 years of occupational exposure to styrene levels of 20 – 30 ppm.

In two studies (57, 87), effects on color vision were shown at exposure levels around 10 ppm, and three other studies (46, 52, 53) showed effects on color vision at exposures of 20 ppm or lower. In three of these five studies, subjects had been exposed for six to seven years.

Some studies have shown effects on hearing at low styrene levels, even when noise levels are factored in. In one study, hearing loss was noted at air levels of 18 ppm (career average), 3.8 ppm (current air levels) and a level of MA in urine corresponding to an air level of >9 ppm. Only age, current noise exposure and MA in urine had a significant effect on hearing in the final multiple logistic regression analysis (112). Another study reports an odds ratio of 3.9 (95% CI: 2.4 – 6.2) for hearing loss with styrene exposure, after adjustment for age, sex, and current and previous noise exposure, and also a significant positive linear correlation between average career exposure to styrene (14.5 ± 12.2 ppm, range 0.8 – 72.5 ppm) and elevated hearing thresholds at 6 and 8 kHz (141, 142). A conference proceedings contains a report of significantly higher hearing thresholds in a small group exposed to about 7.5 ppm styrene (calculated from MA+PGA in urine) than in a control group (106). Deriving an effect level for hearing damage caused by styrene from the information in these studies is problematic, since there are large uncertainties in estimates of previous exposure levels for both styrene and noise, and the occurrence and effect of exposure peaks and impulse sound are unclear. Considered together, however, the studies indicate an effect level of about 10 ppm. Performance on balance tests is affected at higher levels (19, 146).

In the IARC evaluation published in 2002, the evidence for mutagenic effects in people occupationally exposed to styrene was judged to be weak. Well designed and well made studies published since then contain analyses of chromosome damage in large groups of exposed subjects, and have now shown that exposure-related chromosome damage occurs in people occupationally exposed to styrene. Both chromosome aberrations and micronuclei in peripheral lymphocytes have been reported. At an exposure level of 8.7 ± 0.9 ppm, Migliore *et al.* (111) reported significant correlations between chromosome aberrations and levels of styrene in air and 4-vinylphenol in urine of 45 Italian laminators. There was

also a significant correlation between micronuclei in lymphocytes and level of 4-vinylphenol in urine. Significantly elevated levels of micronuclei were observed in two other studies at average exposure levels of 9.5 ppm (55) and 16.8, 17.5 and 19.3 ppm (data from three monitoring sessions) (92).

Electrophilic effects on macromolecules, in the form of hemoglobin adducts, DNA adducts and DNA strand breaks, have been reported at occupational exposure to styrene levels below 20 ppm (55, 92, 155).

Styrene causes lung cancer in mice. Benign lung tumors have been reported in mice after exposures as low as 20 to 40 ppm, and malignant tumors at 160 ppm (37).

The differences between rats, mice and humans with regard to measured and simulated levels of SO, sensitivity to glutathione depletion, and metabolic capacity in the lungs indicate that people are considerably less sensitive than mice to damage to pulmonary epithelium.

With occupational exposure to styrene levels above 20 ppm there was a significantly greater risk for elevated prolactin levels in serum (100). The clinical significance of this is not clear, however. In a multi-generation study with rats, exposure to 150 ppm resulted in lower body weights prior to weaning in the F_2 generation. No effects were seen at 50 ppm (40, 41). At 150 ppm there was some maternal (F_1) toxicity in the form of a not significant weight loss prior to mating.

Conclusions

The critical effects of occupational exposure to styrene are genotoxicity, hearing loss and effects on color vision. Styrene is probably genotoxic to humans and possibly also carcinogenic. Genotoxic effects have been observed at occupational exposures down to about 10 ppm. Effects on color perception have also been documented at occupational exposures around 10 ppm, and hearing loss is presumed to occur at approximately the same levels.

Skin exposure to styrene in liquid form can result in significant uptake.

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Appendix 1

Abbreviations

ALAT	Alanine aminotransferase
ALP	Alkaline phosphatase
ASAT	Aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BAT-value	Biological tolerance value
CC16	Clara cell protein
CI	Confidence interval
CYP	Cytochrome P450
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
GSD	Geometric standard deviation
GSH	Glutathione
GST	Glutathione transferase
HCRA	Howard Center for Risk Analysis
IARC	International Agency for Research on Cancer
IHD	Ischemic heart disease
LDH	Lactate dehydrogenase
M1	N-acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine
M2	N-acetyl-S-(2-phenyl-2-hydroxyethyl)-L-cysteine
MA	Mandelic acid
PGA	Phenylglyoxylic acid
SD	Standard deviation
SMR	Standardized Mortality Ratio
SO	Styrene 7,8-oxide

Consensus Report for Sulfuric, Hydrochloric, Nitric and Phosphoric Acid

June 3, 2009

This Consensus Report is based primarily on the Criteria Document compiled by the Nordic Expert Group (NEG) (85). A final literature search of PubMed and Toxline was made on October 29, 2008.

Chemical and physical data. Occurrence

Identification, description	Sulfuric acid	Hydrochloric acid	Nitric acid	Phosphoric acid
CAS No.:	7664-93-9	7647-01-0	7697-37-2	7664-38-2
Synonyms:	dihydrogen sulfate	hydrogen chloride (liquid), chlorohydric acid	hydrogen nitrate, nitroso nitric acid	orthophosphoric acid
Formula:	H_2SO_4	HCl	HNO_3	H_3PO_4
Mol weight:	98.08	36.46	63.02	98.00
Boiling point (°C):	338 (98%)[a]	110 (20%) - 85 (gas)	121 (70%) 86 (100%)[b]	158 (85%)
Melting point (°C):	10 (100%)	- 85 (25%) - 114 (gas)	- 42 (70%)	26 (100%)
Density (g/ml):	1.84 (100%)	1.19 (38%)	1.41 (70%)	1.71 (85%) 1.86 (100%)
pKa:	- 3.0; 1.99	- 8.0	- 1.3	2.12; 7.21; 12.32
Distribution coefficient[c]:	- 2.20	0.54	0.21	- 0.77
Odor threshold (mg/m^3):	> 1	1 – 50	0.75 – 2.50	Odorless
Conversion factors: (25 °C, 101.3 kPa)				
1 mg/m^3 = 1 ppm =	0.25 ppm 4.0 mg/m^3	0.7 ppm 1.4 mg/m^3	0.4 ppm 2.5 mg/m^3	0.25 ppm 4.1 mg/m^3

[a] Percent by weight in aqueous solution.
[b] Disintegrates.
[c] Octanol/water (log P_{ow}), estimated values.

Sulfuric acid, hydrochloric acid and nitric acid are strong acids that dissociate completely in water at low to moderate concentrations; phosphoric acid is weaker. All four of these acids are hygroscopic (attract water) and corrosive. Nitric acid and relatively concentrated sulfuric acid are also oxidizing.

Aqueous solutions of these acids are not flammable *per se*, but on contact with metals may release highly flammable and explosive hydrogen gas (for nitric acid, on contact with only a few metals).

All four acids are important industrial chemicals with broad areas of use, e.g. in production of chemicals, fertilizers, and paper pulp; for surface treatment of metals; and as pH regulators. Sulfuric acid is also used in batteries and as a drying agent, hydrochloric acid as a disinfectant, and nitric acid in the production of explosives.

Sulfuric acid was previously used in Sweden for pickling steel (cleaning prior to electroplating), but in the late 1980s and early 1990s this use was almost completely phased out in favor of hydrochloric acid or – with production of stainless steel – hydrofluoric acid, either alone or mixed with nitric acid (personal communication, Cecilia Andersson, Swedish Industrial and Chemical Employers Association.

Sulfuric acid and phosphoric acid, which are less volatile than the other two acids, occur in workplace air primarily as aerosols, whereas hydrochloric acid and nitric acid occur more often as vapors. Various types of sorbent tubes and filter cassettes are used for sampling. Analysis is usually made with ion chromatography, but the presence of the acid's salts may cause some interference. Analysis of sulfuric acid is subject to interference from sulfur dioxide, sulfites and organic sulfur compounds as well; moreover, different analysis methods have often led to considerable differences in measured concentrations (14, 36, 37).
The most sensitive analysis methods for sulfuric acid have detection limits of 0.003 and 0.01 mg/m^3 with 8 hours of sampling time (69).

According to the CAREX database, in 1990 – 1993 about 7,900 persons in Sweden were exposed to "aerosols of strong inorganic acids containing sulfuric acid" (16).

Sulfuric acid

Sulfuric acid is a colorless (if pure) to dark brown, oily liquid. It is odorless unless heated, when it releases pungent fumes. It produces heat on reaction with water. On reaction with many organic substances the main residue is carbon.

Concentrated sulfuric acid is 96 – 98% (by weight, 18 M), and battery acid is 33.5%. "Fuming" sulfuric acid, also called oleum, is a solution of sulfur trioxide in anhydrous sulfuric acid.

Production of concentrated sulfuric acid is a closed process until discharging, when some exposure may occur. Few exposure measurements have been made during this stage of the process, however. The most recent measurements with personal monitors are from 2007 and showed levels below 0.04 mg/m^3, which is near the detection limit. On this occasion the charge was somewhat lower than usual. In production of copper, the temperature in the electrolysis bath is 67 – 68 °C and the content of sulfuric acid is 17.5%. Exposure occurs primarily when the electrolyte tanks are being emptied (personal communication, Cecilia Andersson, Swedish Industrial and Chemical Employers Association.

Exposure measurements (n = 134) made in Norway in the 2000 – 2006 period showed average levels of 0.003 – 0.14 mg/m^3 in most industries: levels were higher with production of iron, steel and iron alloys, where the average level for 3 measurements was 3.5 mg/m^3 and the highest value was 10.2 mg/m^3 (85).

Further information on occupational exposure levels is contained in the Nordic Criteria Document (85).

Hydrochloric acid

The term "hydrochloric acid" refers to an aqueous solution of hydrogen chloride gas. This acid, which is colorless and has a sharp odor, is produced in concentrations up to about 38% (by weight, 12 M). A mixture of concentrated hydrochloric acid and a strong oxidant, e.g. a 3:1 mixture of concentrated hydrochloric acid and nitric acid (*aqua regia),* dissolves gold.

Exposure measurements (n = 33) made during 2000 – 2006 in various industries in Norway showed average levels of 0.0033 – 0.23 mg/m^3, with the highest level (1.1 mg/m^3) in science/technology research and development (85). In a Dutch study published in 1982, it was estimated that workers at a zinc galvanizing plant (pickling) were exposed to air levels above 7 mg/m^3 for 27% of the time (64).

Nitric acid

Pure nitric acid is a clear liquid with a characteristic suffocating odor, and in damp air it becomes a white, fuming liquid. Nitric acid is difficult to produce in pure form, since it readily decomposes into water, nitrous oxides and oxygen. Vapors of nitric acid are therefore always a mixture of the acid and its decomposition products. Nitrogen dioxide colors the liquid yellow, shading to red as the concentration rises.

Concentrated nitric acid is about 70% (by weight, 16 M). White fuming nitric acid is extremely concentrated, usually >90%, and contains very little nitrogen dioxide (0.1 – 0.4%). Red fuming nitric acid contains more nitrogen dioxide.

Concentrated nitric acid is a strong oxidizer that reacts, often explosively, with combustible, organic and easily oxidized materials.

Exposure data are sparse. Measurements (n = 36) made in various industries in Norway in 2000 – 2006 showed average levels of 0.013 – 0.061 mg/m^3. The highest measured value was 0.17 mg/m^3 (85). Individual air levels compiled by the IARC, dating mostly from 1975 – 1983, were in the range 0.01 – 2.8 mg/m^3 (38).

Phosphoric acid

"Phosphoric acid" in this document refers to aqueous solutions of the pure acid, which is a solid substance at room temperature and normal atmospheric pressure. An 85% aqueous solution of phosphoric acid is a clear, viscous liquid with neither color nor odor.

Exposure measurements (n = 13) made in various industries in Norway in 2000 – 2006 showed average levels of 0.0003 – 0.74 mg/m^3. The few measurements compiled by the IARC were below 0.67 mg/m^3 (38).

Uptake, distribution, biotransformation, excretion

This section is based on the Nordic Criteria Document (85). Because of their high affinity for water, vapors of hydrochloric acid or nitric acid can be transformed to aerosols in the damp air of the respiratory passages. Aerosols of the four acids are deposited in the respiratory passages as droplets.

The droplet size of an acid aerosol is usually the factor that determines where in the respiratory passages it is deposited, but respiratory rate and way of breathing are also relevant. The particle size of an aerosol is usually given as the diameter of the spherical particle representing the median in the aerosol. The median is based either on the mass of the particle (Mass Median Diameter, MMD) or on the mass of a particle with the density of 1 which has the same sedimentation rate in air as the particle in question (Mass Median Aerodynamic Diameter, MMAD). The median can also be based on the volume (Volume Median Diameter, VMD).

Inert particles with an aerodynamic diameter of 5 – 30 µm are deposited mostly in the nose and throat (nasopharynx) by impaction. Smaller particles sediment in the tracheobronchial region (1 – 5 µm) or are deposited by diffusion in the alveoli (<1 µm).

Hygroscopic droplets attract water and increase in size on their way through the respiratory passages. The growth of sulfuric acid droplets tends to increase retention in the respiratory passages, compared to inert particles of the same size as either the original or the enlarged acid droplets. Sulfuric acid aerosols are deposited mostly in the upper respiratory passages. An important area of deposition is the larynx.

Nitric acid vapor is generally deposited in the upper respiratory passages because of its high solubility in water and high reactivity. If other particles act as vectors, however, the vapor can reach the lower respiratory passages.

Inhaled acid aerosols are neutralized in the upper respiratory passages by the body's own ammonia in the mouth cavity.

The acids are protolyzed, but unless exposure is extremely high the contribution of the protons and anions to the body's total pool is negligible. Locally, however, the concentrations of protons and anions can be high.

There are no data on skin uptake, but the polarity indicates that absorption via intact skin should be low.

Toxic effects

The inhalation toxicity of the four acids seems to be mostly due to the protons liberated by their dissociation. Low pH is believed to be an important factor in the development of tooth erosion, for example. As mentioned previously, nitric acid and relatively concentrated sulfuric acid are oxidizing. All four acids are

classified as corrosive in the European legislation on classification and labeling: hydrochloric acid and phosphoric acid in solutions of 25% or higher, sulfuric acid 15% or higher, and nitric acid as low as 5% (lower limits for classification). Severe reactions, with chemical burns that may cause sores, blindness and even death, are described after contact with skin, eyes and mucous membranes. Acute pulmonary edema has been reported after inhalation of high concentrations, as well as potentially fatal "acute respiratory distress syndrome" (ARDS) and chronic "reactive airways dysfunction syndrome" (RADS). Repeated or prolonged skin exposure to dilute solutions can cause contact eczema (85).

There are no reports of sensitization after exposure to any of these acids (85).

Because of the pronounced dehydrating ability of sulfuric acid and the heat produced on its reaction with water, sulfuric acid causes more tissue damage than might be expected from the strength of the acid (85).

The published LC_{50} values in mmol/m^3 (mg/m^3) are 0.2 – 5.2 (18 – 510) for sulfuric acid, 2.1 – 4.1 (130 – 260) for nitric acid, >8.7 (>850) for phosphoric acid and 21 – 329 (780 – 12,000) for hydrochloric acid (85).

Aside from the cancer studies (see below under Carcinogenicity), there are only a few studies reporting effects of long-term occupational exposure to these acids. Most of the reports are about erosion damage to teeth (85). A survey article concludes (on the basis of prevalence studies with control groups) that workers occupied in the battery industry and with galvanizing/pickling, and who were exposed to sulfuric acid or hydrochloric acid, and to a lesser degree to phosphoric acid, nitric acid and hydrogen fluoride, had an elevated risk of tooth erosion. There was considerable variation in prevalence for both exposed subjects and controls, however: 26 – 100% vs. 0 – 80% (86). The prevalence of tooth erosion varies with age, diet and eating habits, and is not particularly well known in the general population. A survey article reports prevalence figures of 4 – 82% for adults aged 18 to 88 (41).

The toxicological literature for each of these acids is summarized in the following sections. More detailed descriptions of individual studies and information on effects at higher exposure levels are presented in the Nordic Expert Group's Criteria Document (85).

Sulfuric acid: Experimental studies with human subjects

Several studies have been made with volunteers, both healthy and asthmatic. The studies differ with regard to exposure method (chamber, mouthpieces or nasal masks), exposure levels (0.01 – 3 mg/m^3), droplet size (0.1 – 10 µm MD), relative humidity (<10 – 100%), exposure time (10 minutes – 6.5 hours), degree of physical activity, and control of levels of endogenous ammonia in the mouth cavity. The subjects served as their own controls. The control conditions were usually pure air or an aerosol of a physiological saline solution. Some of these studies are described below. The results from combined exposures are included only in exceptional cases.

Lung function: Mucociliary clearance and other pulmonary defense mechanisms

The effect of sulfuric acid aerosols on mucociliary clearance has been examined in several studies. In the usual experimental design, the subjects inhaled a radioactively labeled inert aerosol (e.g. ^{99m}Tc-labeled Fe_2O_3 or ^{85}Sr-labeled latex particles) for a few minutes prior to their exposure to the sulfuric acid. The emitted γ-radiation was then monitored with a series of measurements made over a number of hours, sometimes with a final measurement 24 hours after the exposure. In several studies, an effect on bronchial mucociliary clearance (either stimulation or inhibition) was seen in healthy volunteers after 1 hour of exposure to 0.1 – 0.47 mg/m^3 or 1 mg/m^3 (48 – 50, 60, 74, 75). An effect at 0.1 mg/m^3 was demonstrated in three studies (49, 50, 75). An effect on tracheal mucociliary clearance in healthy volunteers was shown in only one study with exposure to larger droplets (MMAD 10.3 µm) at 0.47 mg/m^3 (48). The mechanism behind the effect on mucociliary clearance is not clear, but changes in pH and viscosity, for example, have been shown and may be relevant (84).

The effect on clearance can be either stimulating or inhibiting, and seems to depend on the acid concentration and the size of both the sulfuric acid droplets and the inert test particles that the clearance is calculated from. The proposed explanation for the apparently contradictory effects attributes them to differences in dosimetry; i.e. that short-term exposures to low concentrations have a stimulating effect, whereas high exposure or prolonged exposure to low levels can damage the epithelium and inhibit clearance.

Accordingly, in a study with 10 healthy volunteers, bronchial clearance of iron oxide particles (MMAD 7.5 µm) was faster after 1 hour of exposure to 0.1 mg/m^3 (MMAD 0.5 µm) than after the control exposure. With exposure to 0.3 mg/m^3 the responses of the subjects ranged from marked acceleration to marked retardation, and at 1 mg/m^3 clearance was reduced (49). In a follow-up study with the same experimental design (8 subjects), but with smaller iron oxide particles (MMAD 4.2 µm), clearance was reduced after all exposures. The authors' explanation for this was that the deposition pattern of the smaller iron oxide particles more closely resembles that of the acid (50). In another study, reduced clearance (iron oxide, MMAD 5.2 µm) was observed in healthy subjects (n = 10) after exposure to 0.1 mg/m^3 (MMAD 0.5 µm) for 1 or 2 hours (75).

Reduced superoxide anion production and reduced adhesion ability were seen *ex vivo* in pulmonary macrophages from bronchoalveolar lavage of 12 healthy subjects after exposure to 1 mg/m^3 (MMAD 0.9 µm) for 3 hours. There was no observed effect on viability or phagocytosis (89). In another study with similar design and exposure level, there were no observed effects on function of alveolar macrophages or antimicrobial defense and no indications of inflammation in bronchoalveolar lavage fluid (26).

Lung function: other effects

Hyperreactivity in respiratory passages after provocation with carbachol was demonstrated in 14 healthy subjects after 16 minutes of exposure to 1 mg sulfuric acid/m^3 (MMAD 0.6 – 1 µm). The subjects inhaled carbachol (1%) with or without previous exposure (16 minutes) to sulfuric acid or to a saline aerosol (1%). Lung function was measured both before and after exposure. After the exposure to sulfuric acid, specific airway conductance was reduced by about 24% (read from the figure), compared with about 9 – 10% after inhalation of carbachol alone as well as after inhalation of the saline aerosol followed by the carbachol. Maximum expiratory flow at 60% of total lung capacity was also lower ($p < 0.05$) after exposure to the sulfuric acid than it was after the other exposures. The effect was not seen after exposure to 0.1 mg/m^3 sulfuric acid (82). In other studies, single exposures to sulfuric acid in concentrations of 0.1 – 0.47 mg/m^3 and 1 – 2 mg/m^3 had no effect on lung function of healthy volunteers (3, 7 – 9, 26, 48 – 51, 66, 74, 75, 80). However, some studies (described below) indicate that asthmatics and perhaps especially young people are more sensitive. A small (reversible) but significant reduction in FEV_1 (forced expiratory volume during one second) was seen in 14 young asthmatics (aged 13 – 18) after 45 minutes of exposure to 0.035 mg/m^3 (MMAD 0.6 µm) compared with the change after exposure to air. A tendency to lower FEV_1 was also observed after exposure to 0.07 mg/m^3 for the same amount of time, but the effect was not observed after 90 minutes of exposure at these levels (46). In another study by the same research team, small (reversible) but significant reductions are reported in FEV_1, total respiratory resistance and forced expiratory flow at 50% of forced vital capacity (FEF_{50}) in 10 young asthmatics (aged 12 – 17) exposed for 40 minutes to 0.1 mg/m^3 (MD 0.6 µm) (45).

Adult asthmatics had poorer lung function (lower FEV_1) after exposure during exercise to 0.35 mg/m^3 (MMAD 0.8 µm) sulfuric acid in combination with low levels of ammonia in the mouth (obtained by gargling with a lemon concentrate and impedes neutralization of the acid) (83). The same research team also reported increased bronchoconstriction (reduced specific airway conductance) and bronchial hyperreactivity on provocation with carbachol in adult asthmatics after 16 minutes of exposure during rest to 0.45 mg/m^3. Exposure to 1 mg/m^3 (MMAD 0.6 – 1 µm) caused reduction in FEV_1 as well (81, 82). Other studies report no observed effect on lung function of adult asthmatics exposed to up to 1 mg/m^3 or higher (4, 66, 80).

Symptoms

Single exposures to 0.1 – 0.47 mg/m^3 (MMAD 0.1 – 10 µm) had no effect on subjective symptom assessments made by healthy volunteers (3, 7, 8, 26, 45, 48, 51, 80). However, a dose-dependent increase was seen in subjective assessments of respiratory symptoms made after one hour of exposure to larger droplets (VMD 10 µm) at 0.5 – 1.5 mg/m^3 (9), although exposure to smaller droplets (MMAD 0.9 µm) at 1 – 1.5 mg/m^3 had no effect on the subjective symptom assessments (8).

In the above study, asthmatics reported no increase in symptoms at 0.5 mg/m^3 (large droplets, VMD 10 µm), but reported an increase at exposure to 1 mg/m^3 (MD 0.9 and 10 µm) (8, 9).

Other studies with healthy and asthmatic subjects report no effects at 2 – 3 mg/m^3 (4, 80).

Sulfuric acid: Studies of occupationally exposed subjects

Exposures are poorly described in the epidemiological studies. Further, the measurements are of questionable validity since results vary with sampling method and are subject to interference from the presence of both inorganic and organic sulfur compounds. Information on droplet size in industrial aerosols is usually lacking, but the median diameter (MD) can be up to 14 µm. In a study made at five lead acid battery plants the MD ranged from 2.6 to 10 µm (43).

A conference proceedings from Australia reports irritation of eyes and respiratory passages in workers exposed to 0.1 – 0.5 mg/m^3. Significant differences were seen in symptom reports (self-administered questionnaires) when workers exposed to levels up to 0.15 mg/m^3 (n = 37) were compared to a group exposed to 0.15 – 0.50 mg/m^3 (n = 45). One or more symptoms were reported by 54% and 93% in the two exposed groups, compared to 25% of controls. The symptoms most commonly reported by all the workers were sneezing, coughing, nasal irritation and runny noses. Workers below the age of 40 reported more symptoms than older workers (25).

A cross-sectional study of 225 workers at five battery plants reports that acute work-related symptoms involving e.g. skin, eyes or respiratory passages were no more common in the group with highest exposure (above 0.3 mg/m^3) than in the group with lowest exposure (below 0.07 mg/m^3). Changes in lung function during the day showed no correlation to air levels of sulfuric acid (27). The same population (n = 248) was also examined for effects of long-term exposure. No correlation between respiratory symptoms (cough, mucus, breathlessness, wheezing) and exposure to sulfuric acid was observed. However, forced vital capacity (FVC) was lower in the group with cumulative exposures above 15 mg/m^3 x months (average 0.21 mg/m^3) than in a group with cumulative exposures below 7 mg/m^3 x months (average 0.10 mg/m^3). Tooth erosion was observed in 38% of the workers in the group with highest exposure, compared to 8% in the group with lowest exposure ($p<0.005$ after correction for age and smoking). The earliest cases of "etching" and tooth erosion were seen after 4 and 30 months, respectively, of exposure to an average level of 0.23 mg/m^3 (28). Other studies have also mentioned tooth erosion after high or unknown exposures (85).

Nasal symptoms and changes in the cells of nasal mucosa were examined in 52 workers (in 5 factories) with an average exposure time of 6 years. Five days of measurements with stationary monitors showed average air levels of 0.035 – 2.1 mg/m^3. Pale areas and sores were seen only in workers with exposures above 0.2 mg/m^3. Histopathological examination of nasal mucosa revealed that changes such as squamous cell metaplasia, squamous atypia and slight dysplasia were

more common among exposed workers than among controls (29). This study has shortcomings in both design and reporting (description of the control group, data processing, statistical analysis etc.).

Sulfuric acid: Animal studies

In general, the results of animal studies support the results of the human studies. Irritation and effects on lung function and pulmonary defense mechanisms (clearance, phagocytosis, production or release of superoxide anions, hydrogen peroxide and mediators such as TNFα). The effects on lungs appear at about the same exposure levels in animals as in humans. Changes in cells of the respiratory epithelium (hyperplasia) have been reported after repeated exposure to 0.125 mg/m^3 (rabbits) and 0.38 mg/m^3 (monkeys) (85).

Proliferative changes were observed in cells in rat larynx after exposure to 1.38 and 5.52 mg/m^3 for 5 or 28 days. At 0.3 mg/m^3, minimal squamous cell metaplasia was observed in the larynx after 28 days. No histopathological changes related to sulfuric acid were observed in lungs or nose at any of the exposures (44). The response to 0.3 mg/m^3 can be regarded as adaptive, and implies that with prolonged exposure there is a risk of cell changes in respiratory epithelium.

Hydrochloric acid

Ten asthmatics (aged 18 to 25) showed no more airway irritation, effect on lung function, fatigue, headache or dizziness after 45 minutes of exposure via half-face mask to 1.1 or 2.5 mg/m^3 than they did after exposure to air alone (78). The authors also compiled information from older human studies and case reports, which indicated that the odor threshold for hydrochloric acid is in the interval 1.5 – 7.5 mg/m^3; that work can be done unhindered at 15 mg/m^3, is difficult at 15 – 75 mg/m^3, and unbearable at 75 – 150 mg/m^3; and that 1,950 – 3,000 mg/m^3 are lethal concentrations (78).

Workers (number not given) in a steel pickling plant showed no evidence of mucous membrane irritation at 3 – 4.5 mg/m^3. Initial, mild and brief irritation of respiratory passages was observed at about 5 mg/m^3, slight irritation at 7 – 11 mg/m^3 and breathing difficulty at 26 – 34 mg/m^3. Chronic bronchitis was also reported after several years of exposure at the latter levels. It was also noted that no effects on the teeth appeared at average concentrations of 4.5 – 7.7 mg/m^3 (53). This study is based on many years of observation, but does not meet modern standards for scientific documentation.

A high prevalence of tooth erosion was seen in a study of industrial workers exposed to hydrochloric acid, but the study reports no air levels (79). In another study, the prevalence of tooth erosion was 90% among 38 workers in a zinc galvanizing plant, but since there was no control group this result is difficult to interpret. Exposure measurements made during 6 workshifts showed average levels of 1.8 – 12.4 mg/m^3 (geometric means) (64).

Guinea pigs exposed to 15 mg/m^3 2 hours/day, 5 days/week for 7 weeks showed no effects on lung function and no histological changes in lungs or respiratory passages (61). The RD_{50} (the exposure level at which respiratory rate is lowered by 50%) for mice is reported to be 309 ppm (432 mg/m^3) with exposure 6 hours/day. After three exposures all the animals were dead or dying (15).

In a 90-day study with mice and rats, no systemic effects could be seen at 30 mg/m^3, although at 15 mg/m^3 – the lowest exposure level – nasal irritation was observed in the rats (industry report cited in the MAK documentation, Reference 22).

No nasal cancer was observed in rats after lifetime exposure to 14 mg/m^3 (the only dose level tested), but there were elevated incidences of hyperplasia in larynx and trachea (22% and 26%, vs. 2% and 6% in controls) (70). The purpose of this study was to examine the effect of combined exposure to formaldehyde and hydrochloric acid, not to investigate the carcinogenicity of hydrochloric acid.

In an eye irritation study with rabbits, made in accordance with OECD guidelines, 10% hydrochloric acid was placed in the category "risk of serious damage to eyes" (40).

Nitric acid

No effects on pulmonary function were observed in 28 young asthmatics (aged 12 – 19) after 90 minutes of inhaling a combination of 0.05 ppm (0.125 mg/m^3) nitric acid, 0.12 ppm ozone and 0.30 ppm nitrogen dioxide (47).

Exposure of 9 healthy subjects to 0.2 mg/m^3 for 2 hours, 100 minutes of which was during exercise, had no discernible effects on lung function (FEV_1, FVC, specific airway resistance) or subjective symptom assessments, and there were no effects on indicators of damage or inflammation in the respiratory passages (bronchoalveolar lavage 18 hours after the exposure) when compared to the effects of exposure to air alone. Alveolar macrophages in the lavage fluid (*ex vivo*) showed more phagocytic activity and greater resistance to infection by RS virus, whereas superoxide anion production dropped (12).

No effects on subjective symptom assessments or inflammatory response and no indications of lung damage (examined with lung function measurements, lavage and bronchial biopsy) were observed in 10 healthy subjects after 4 hours of exposure to 0.5 mg/m^3 (vapor). The subjects were their own controls (6). In a previous study by the same research group, in which 10 healthy volunteers were exposed for 2 hours to nitric acid fog (0.4 mg/m^3, VMD 6 µm), lung function and symptom assessments were the same as after exposure to fog (water aerosol) or ordinary air (5).

In an older study based on 1 or 2 subjects, exposure to 11 – 12 ppm (27 – 30 mg/m^3) for more than one hour was reported to be unbearable and hazardous to human health. Exposure to 84 ppm (210 mg/m^3) could be endured for only two or three minutes (thesis from 1907, cited in the MAK documentation, Reference 21).

The lowest effect levels reported in animal studies pertain to effects on pulmonary defense mechanisms. When rabbits were exposed to vapor of nitric acid

(0.05, 0.15 or 0.45 mg/m^3, 4 weeks) there were effects on alveolar macrophages *ex vivo* (reduced production of superoxide anions) at all tested concentrations, and reduced bronchial reactivity on provocation with acetylcholine and histamine at 0.15 and 0.45 mg/m^3. A dose-related reduction of TNFα release/activity, significant at the two higher exposures, was also reported (68).

In another study, rats were exposed to 0.25 mg/m^3 (vapor) 4 hours/day for 4 days, or to 1 mg/m^3 for 4 hours (same total dose). At both exposures, ability to inhibit elastase activity was enhanced in bronchial lavage *ex vivo,* and the repeated exposure also reduced production of superoxide anions (59).

Chronic exposure of rats to low levels of nitric acid vapor (0.05 mg/m^3, 40 weeks) had no effect on body weight, polyamine levels in lungs, or lung clearance. However, there were elevated levels of a stress protein in the lungs (stress-inducible heat-shock protein 70) (52, 73, 87).

No bronchoconstriction (reduced specific pulmonary flow resistance) was observed in either normal or sensitized sheep (bronchospasm on provocation with roundworm extract) after 4 hours of exposure to nitric acid vapor (4.1 mg/m^3). However, after the exposure the sensitized animals had greater hyperreactivity in respiratory passages on provocation with carbachol in aerosol form (1).

Phosphoric acid

This section takes up only studies in which the effects of exposure to phosphoric acid alone were examined: studies of phosphorus compounds such as phosphorus pentoxide and red phosphorus, which can be transformed to phosphoric acid, are excluded.

Phosphoric acid is a mild irritant to eyes, upper respiratory passages and skin. The dust is more irritating to skin in the presence of moisture (85).

Case reports describe severe effects such as RADS, hyperphosphatemia, hypocalcemia, systemic metabolic acidosis and even death, after massive exposure to phosphoric acid (85).

The LC_{50} for rats was above 850 mg/m^3 with one hour of exposure. No other toxic effects were reported (65).

No other peer-reviewed studies of phosphoric acid were found.

Genotoxicity

Low pH had no effect on the frequency of point mutations in various strains of bacteria and yeast (38), but other genotoxic effects of low pH have been reported in several *in vitro* studies. DNA damage (strand breaks) and effects on chromosomes have been shown in studies of mammalian and eukaryotic cells (13, 17, 38, 42, 54 – 57, 88). The mechanism is not clear, but it has been proposed that low pH causes oxidative stress (42) or induces DNA damage mediated by an inhibition of the enzyme topoisomerase II (TOP 2) (88), which may have a co-initiating effect (32). In cell cultures, low pH has been shown to enhance the expression of genes involved in inflammation, proliferation, differentation, and response to DNA

damage (23), and has also been shown to affect cell proliferation (90). It has been proposed that these effects are important to the development of cancer in the esophagus. Reduced pH functioned as a promotor in a classical skin-tumor promotion test with mice in which DMBA was used as the initiator (88).

Inhalation of the air levels that occur in work environments may cause a local lowering of pH in the respiratory passages, but is hardly likely to affect systemic pH.

Carcinogenicity

In 1992, the IARC classified "aerosols of strong inorganic acids containig sulfuric acid" as carcinogenic to humans (Group 1) (38). The classification is based primarily on cohort and case-control studies showing correlations between long-term exposure and cancer in respiratory passages, especially the larynx. The exposed subjects in these studies worked in the metal industry (pickling) or petrochemical industry, or in production of isopropanol, soap or fertilizer. Exposure in these studies is generally not well described, but there are exposure data for a cohort of steelworkers. The cohort consisted of persons who had worked with pickling for at least 6 months in the 1940 – 1965 period (average start of employment was 1949). Sulfuric acid was the predominant acid in the pickling process until the mid-1960s. According to a classification made by occupational hygienists, 62% of the workers had been exposed to sulfuric acid only, 22% to sulfuric acid and other acids, and 16% to other acids only. Exposure to sulfuric acid had stopped by the time the study began. Exposure data from two of the three plants, covering the 1975 – 1979 period, showed average sulfuric acid exposure levels of 0.19 mg/m^3 (personal monitors, n = 15) and 0.29 mg/m^3 (stationary monitors, n = 34). The authors pointed out that, although the production process had not been changed, it was still possible that exposures were higher prior to1975 due to a lower awareness of the risks (11, 76, 77). The cohort (n = 1,065) was first studied for mortality due to lung cancer (11). Later, 77% of the study population was examined for incidence of laryngeal cancer (76). After the IARC assessment, the incidence cohort was followed up (77). The length of exposure for this group on the two study occasions was on average 9.2 – 9.5 years. In the follow-up 14 exposed cases were found, 7 of which were exposed to sulfuric acid only, 4 to sulfuric acid and other acids, and 3 to other acids only. The relative risk of laryngeal cancer for all the exposed subjects was 2.2 (95% Confidence Interval: 1.2 – 3.7) compared with the national average and after correction for use of tobacco and alcohol.

In a cohort (n = 2,678) of battery manufacturers and steelworkers, exposed primarily to sulfuric acid but also to hydrochloric acid, mortalities (SMR) due to all cancers and to cancers of the larynx or lungs were lower than the national averages. A case-control study (n = 15) based on the same cohort yielded no definite information on "upper aerodigestive" cancers, including lip cancer, because there were too few cases (OR 2.0; 95% CI: 0.4 – 10 for cases with at least 5 years of exposure) (20). There is no information on use of tobacco or

alcohol. Hathaway later pointed out other shortcomings in the study, such as that the odds ratio would be close to 1 if the four cases of lip cancer were excluded from the analysis (31).

No increases in mortality due to laryngeal cancer (4 cases vs. 3.1 expected) or lung cancer (27 vs. 32.8) were observed in a cohort of 1,409 workers in a plant for production of sulfuric acid. Nearly half (46%) of the workers had previously worked in mines. An elevation in mortality due to myeloid leukemia could not be explained by the exposure (63).

There are reports of one case of laryngeal cancer and three cases of naso-pharyngeal cancer attributed to sulfuric acid exposure (34, 35).

A population-based case-control study (included in the IARC monograph) indicated an elevated risk of esophageal cancer after exposure to sulfuric acid ($p < 0.10$) (72, also published in Reference 62).

In a recently published case-control study of workers at a nickel refinery who were exposed to low levels of sulfuric acid (usually below 0.5 mg/m^3), no elevation in risk of lung cancer caused by the acid was seen (30).

Cocco *et al.* made a case-control study of stomach cancer based on death certificates from 24 states in the U.S. No elevation in risk was correlated to occupational exposure to sulfuric acid (19). In another case-control study (n = 1,056) based on death certificates, the same research group evaluated the risk of gastric cardia cancer in various jobs and industries. Exposure was divided in-to three categories according to intensity but was not numerically quantified. Among white males there was a significant increase in risk with increased exposure to sulfuric acid (18).

Historical exposure levels (prior to 1970) of sulfuric acid are described in a survey article. Exposures (8-hour averages) were estimated to have been above 1 mg/m^3 in production of sulfuric acid and isopropanol and around pickling of metals, 0.1 – 1 mg/m^3 in e.g. soap production, and below 0.1 mg/m^3 in refining copper and zinc and in production of phosphate fertilizers and lead acid batteries. Beginning in the early 1970s various measures have been introduced which have lowered exposure levels considerably (67). Measurements of sulfuric acid made (without reference to cancer occurrence) in pickling plants in the 1970s and 1980s and compiled by the IARC showed that the average level in most cases was 0.l – 0.3 mg/m^3, with a highest average of 3 mg/m^3 (38).

In summary, the uncertainty of exposure data for sulfuric acid in the epidemiological studies, in combination with the analytic difficulties previously encountered in monitoring sulfuric acid, make it impossible to correlate an increase in cancer incidence to an exposure level (69). The occurrence of cancer is apparently secondary to the severe, chronic local irritation and the damage to respiratory epithelium due to the lower pH caused by the acid. SCOEL and DECOS present the hypothesis that there is a threshold for the genotoxic effect (33, 69).

In its 1992 assessment, the IARC placed hydrochloric acid in Group 3: “not classifiable with regard to carcinogenicity to humans” (39). The assessment was based on about 10 studies.

A recently published case-control study of workers exposed to inorganic acid aerosols (mostly hydrochloric acid but also nitric acid, phosphoric acid, sulfuric acid, hydrogen fluoride and chromic acid) reports no elevation in risk for laryngeal or hypopharyngeal (lower throat) cancer. A significant elevation in risk for cancer in the hypopharynx (4 exposed cases) was observed with exposures exceeding 15 years, but no correlation was seen between cumulative exposure and cancer incidence (71).

The population-based case-control study mentioned above (72) indicated elevated risk ($p < 0.10$) for cancer in the pancreas (n = 5), prostate (n = 9) and kidneys (n = 4) after exposure to nitric acid. For phosphoric acid, the analyses showed elevated risks for renal cancer (n = 6) and lung cancer (n = 14) (72).

Effects on reproduction

In the only available study on reproduction effects, no embryotoxic, fetotoxic or teratogenic effects were observed in mice and white rabbits exposed to sulfuric acid during the organogenesis period of gestation (0, 5 or 20 mg/m^3, 7 hours/day). Slight maternal toxicity was observed in both species at the higher exposure level (58).

It is unlikely that occupational exposure to any of these four acids has toxic effects on reproduction because their contribution of protons and anions to the body's pool is negligible, but secondary effects due to lung damage are a possibility.

Dose-effect/dose-response relationships

Dose-effect relationships observed with inhalation exposure to sulfuric acid, hydrochloric acid and nitric acid are summarized in Tables 1 – 3. There are no relevant studies of phosphoric acid. The tables list the lowest exposure level having a particular effect (LOAEL, lowest observed adverse effect level) and the highest level that did not have an effect (NOAEL, no observed adverse effect level). There are no data on skin uptake for any of these four acids, but their polarity suggests that absorption is minimal if the skin barrier is intact.

Sulfuric acid

The toxicological data from short-term and long-term studies of animals and humans are relatively consistent. In many studies the effects have appeared at the lowest tested exposure level. With present analysis methods, it is possible to make reliable measurements of sulfuric acid at the lowest effect levels with 8-hour samples, provided there are no interfering substances in the air, but shorter sampling times are still problematical (69).

The effects observed at the lowest exposure levels are effects on lung function and pulmonary defense mechanisms, as well as irritation. Effects on bronchial mucociliary clearance have been reported in studies of healthy subjects exposed to 0.1 mg/m^3 (49, 50, 75). One study of occupationally exposed subjects indicated

that irritation of eyes and respiratory passages begins to appear at about 0.1 mg/m^3 (25), although another field study reports no irritation at somewhat higher levels (27). Healthy volunteers did not report irritation at levels below 0.5 mg/m^3 (9). In one study, hyperreactivity in respiratory passages (carbachol) was observed in healthy volunteers exposed to 1 mg/m^3 (82), whereas other studies report no effect on lung function at that level. Some studies indicate, however, that asthmatics – especially young asthmatics – may be more sensitive. Small declines in lung function have been reported in young asthmatics exposed during exercise to 0.1 mg/m^3 or even lower concentrations (45, 46). The lowest exposure level having effect on lung function of adult asthmatics is 0.35 mg/m^3 in combination with low levels of endogenous ammonia in the mouth cavity (83). Tooth erosion, declines in lung function and cell changes in nasal mucous membranes have been reported at occupational exposures of about 0.2 mg/m^3 (28, 29).

The results from animal experiments support the evidence from human studies. Irritation, effects on clearance and other pulmonary defense mechanisms and on lung function, and cell changes in respiratory epithelium have been observed. Effects in animals have been reported at about the same exposure levels as in humans.

Occupational exposure to aerosols of strong inorganic acids containing sulfuric acid has been shown to cause cancer in respiratory passages. Exposures in the epidemiological studies are in general poorly described, but elevated cancer incidence was observed in a cohort with a reported average exposure level of 0.2 mg/m^3 (11, 76, 77). However, exposure began long before measurements were made and it is probable that levels were previously higher. The analytical problems encountered with measuring sulfuric acid further increase the uncertainty in the exposure estimates. It is therefore not possible to identify a level that yields an increase in cancer risk. Available data indicate that development of cancer may involve indirect DNA damage, oxidative stress and cytotoxicity. It is reasonable to assume that DNA damage does not appear below the threshold dose at which the cell's buffering capacity is exceeded.

Hydrochloric acid

Toxicological data are sparse. There were no changes in lung function, no respiratory symptoms, and no fatigue, headaches or dizziness in asthmatic adults exposed to up to 2.5 mg/m^3 for 45 minites (78).

In a study of occupationally exposed workers, it was noted that no effects on teeth appeared at average concentrations of 4.5 – 7.7 mg/m^3 (no further information is given). No irritation was observed at 3 – 4.5 mg/m^3; slight, brief respiratory irritation at about 5 mg/m^3; slight irritation at 7 – 11 mg/m^3; and breathing difficulty at 26 – 34 mg/m^3. Chronic bronchitis was also reported after several years of exposure at the latter levels (53). This study was based on several years of observations, but the study design and documentation do not meet modern requirements.

An elevated incidence of hyperplasia in larynx and trachea was observed in rats after lifetime exposure to 14 mg/m^3 (the only exposure level tested) (70). At about the same level (15 mg/m^3, 90 days) nasal irritation has been reported in rats (industry report cited in the MAK documentation, Reference 22).

Nitric acid

Healthy subjects were exposed to 0.2 mg/m^3 for 2 hours, 100 minutes of which was during exercise: the exposure had no effect on lung function, subjective symptom assessments, or markers for inflammation or damage in respiratory passages. Pulmonary defense was stimulated in alveolar macrophages (increased phagocyte activity and greater resistance to infection), but superoxide anion production dropped (12).

No effects were reported in healthy subjects after 4 hours of exposure to 0.5 mg/m^3 (no symptoms, effects on lung function or damage to respiratory passages) or 2 hours of exposure to 0.4 mg/m^3 (no symptoms or effects on lung function) (5, 6).

The lowest effect levels reported in animal studies refer to effects on pulmonary defense mechanisms. At 0.05 – 0.25 mg/m^3 there was an increased ability to inhibit elastase activity in lung lavage *ex vivo*, a reduced level or production of superoxide anions (59), reduced release/activity of TNFα by stimulated macrophages, reduced bronchial reactivity on provocation with acetylcholine and histamine (68), and elevated levels of stress protein in lungs (stress-inducible heat-shock protein 70) (52, 73, 87).

No bronchoconstriction was observed in either normal or sensitized sheep after 4 hours of exposure to nitric acid vapor (4.1 mg/m^3). However, after the exposure the respiratory passages of the sensitized animals showed greater hyperreactivity on provocation with a carbachol aerosol (1).

Judging from animal data on acute toxicity, nitric acid seems to have about the same potency as sulfuric acid.

Phosphoric acid

Phosphoric acid has an LC_{50} above 850 mg/m^3 (>8.7 mmol/m^3). There is no other available information on dose-effect or dose-response relationships.

According to a criteria document published in 1992 by the European Commission, aerosols of phosphorus pentoxide are probably not irritating at concentrations in the range 0.8 – 5.4 mg/m^3 (based on unpublished data contained in the ACGIH documentation, Reference 2). It was also observed that phosphorus pentoxide is a powerful dehydrating agent that reacts with moisture in the air in respiratory passages, forming phosphoric acid and generating heat. Phosphorus pentoxide therefore probably causes more tissue damage than phosphoric acid alone. Exposure limits based on data for phosphorus pentoxide should therefore provide an adequate margin of safety (24).

Conclusions

Sulfuric acid

The critical effects of occupational exposure to sulfuric acid are judged to be effects on bronchial mucociliary clearance and lung function, as well as irritation of eyes and respiratory passages. These effects are seen at about 0.1 mg/m^3. At somewhat higher levels (about 0.2 mg/m^3) tooth erosion and pathological changes in nasal mucosa have been reported in exposed workers.

Damage to respiratory epithelium and cancer (see below) probably do not occur below the levels that affect mucociliary clearance and cause irritation.

Hydrochloric acid

There are no data from which to derive a critical effect of occupational exposure. No respiratory tract irritation was seen in asthmatics exposed to 2.5 mg/m^3 for 45 minutes. A report that is based on many years of observations of occupationally exposed workers but does not meet modern requirements for a scientific study indicated slight, brief irritation at 5 mg/m^3. Rats exposed to 14 mg/m^3 showed hyperplasia (increase in number of cells) in trachea and larynx.

Nitric acid

In general, there are no data on which to base a critical effect of occupational exposure to nitric acid. Single exposures of healthy volunteers to 0.4 and 0.5 mg/m^3 had no effects on self-reported symptom assessments or lung function, and yielded no indication of inflammatory response in lungs. With a similar exposure to 0.2 mg/m^3 both stimulation and inhibition of activity was observed in alveolar macrophages.

Phosphoric acid

There are no data from which to derive a critical effect for occupational exposure to phosphoric acid.

Carcinogenicity

Epidemiological studies have shown that aerosols of strong inorganic acids containing sulfuric acid cause cancer in respiratory passages. It is not possible, on the basis of these studies, to determine an exposure level at which cancer appears. Development of cancer seems to occur via indirect DNA damage, oxidative stress and cytotoxicity. It is reasonable to assume, however, that DNA damage does not occur until the cell's buffering capacity has been exceeded.

Data on the other acids are not sufficient to support any conclusions regarding their carcinogenic potential.

Table 1. No Observed Adverse Effect Level (NOAEL) and Lowest Observed Adverse Effect Level (LOAEL) in humans subjects exposed by inhalation to low concentrations of sulfuric acid.

NOAEL (mg/m^3)	LOAEL (mg/m^3)	Subjects[a]	Exposure time	Effects	Ref.
–	0.035 and 0.07	Asthmatic adolescents	45 and 90 minutes	Marginal reduction in FEV_1 immediately after 45-minute exposure, but not after 90 minutes. Values normalized 20 minutes after exposure.	46
–	0.1	Asthmatic adolescents	40 minutes including exercise	Decline in lung function (reversible reductions in FEF_{50}, FEV_1 and total respiratory resistance).	45
–	0.1	Healthy volunteers	1 hour	Increase in bronchial mucociliary clearance of larger test particles (MMAD 7.5 µm).	49
–	0.1	Healthy volunteers	1 - 2 hours	Decline in bronchial mucociliary clearance of smaller test particles (MMAD 4-5 µm).	50, 75
–	~0.1	Exposed workers		Irritation of eyes and respiratory passages.	25
–	~0.2	Exposed workers	10 years (mean)	Tooth erosion, reduced FVC.	28
–	>0.2	Exposed workers	6 years (mean)	Cell changes in nasal mucosa.	29
0.35 (high oral level of NH_3)	0.35 (low oral level of NH_3)	Asthmatics	20 minutes rest + 10 minutes exercise	Decline in lung function (reduced FEV_1 and maximum expiratory flow at 60% of total lung capacity).	83
0.1	0.45	Asthmatics	16 minutes	Bronchoconstriction (lower specific airway conductance) and bronchial hyperreactivity (carbachol).	81, 82
–	0.47	Healthy volunteers	1 hour including exercise	Accelerated mucociliary clearance in trachea and small airways.	48
0.47 (MMAD 10 µm)	0.5 (VMD 10 µm)	Healthy volunteers	1 hour including exercise	Small increase of upper and lower respiratory symptoms.	9, 48
0.38 (MMAD 0.9 µm)	1 (MMAD 0.9 µm)	Asthmatics	1 hour including exercise	Increase in reported symptoms (lower respiratory passages, headache, fatigue, eye irritation).	8
0.5 (VMD 10 µm)	1 (VMD 10 µm)	Asthmatics	1 hour including exercise	Increase in reported symptoms (upper and lower respiratory passages).	9
0.3	1	Asthmatics	1 hour	Decline in bronchial mucociliary clearance.	74

Table 1. Continued.

NOAEL (mg/m^3)	LOAEL (mg/m^3)	Subjects[a]	Exposure time	Effects	Ref.
0.1	1	Healthy volunteers	16 minutes	Hyperreactivity in airways on provocation with carbachol.	82
–	1	Healthy volunteers	3 hours including exercise	Lower superoxide anion production by pulmonary macrophages and lower ability to adhere to a substrate.	89

FEV_1 = forced expiratory volume during one second; FEF_{50} = forced expiratory flow at 50% of FVC; FVC = forced vital capacity; MMAD = mass median aerodynamic diameter; VMD = volume median diameter.
[a] The subjects were adults unless otherwise described.

Table 2. NOAELs and LOAELs reported in inhalation studies with low levels of hydrochloric acid.

NOAEL (mg/m^3)	LOAEL (mg/m^3)	Subjects/ Species	Exposure time	Effect	Ref.
Human studies					
4.5-7.7[a]	1.8-12.4	Exposed workers		Tooth erosion (no controls).	53, 64
2.5		Asthmatics	45 minutes	No airway irritation, fatigue, headache, dizziness; no effect on lung function.	78
3-4.5[a]	5.2[a]	Exposed workers		Brief initial airway irritation.	53
–	26-34[a]	Exposed workers		Breathing difficulty, chronic bronchitis.	53
Animal studies					
2.8[b]	14	Rat	6 hrs/day, 5 days/week lifelong	Elevated incidence of hyperplasia in larynx and trachea.	70
–	15	Rat	6 hrs/day, 5 days/week 90 days	Nasal irritation.	22[c]
–	56	Mouse	10 minutes	10% reduction in respiratory rate. RD_{50} = 432 mg/m^3.	10
15	–	Guinea pig	2 hrs/day, 5 days/week 7 weeks	No histological changes in lungs or airways. No effect on lung function.	61

RD_{50} = The exposure level at which respiratory rate is reduced by 50%.
[a] No details are given.
[b] Calculated by the German MAK committee using linear interpolation between the incidence at 0 and at 14 mg/m^3.
[c] Chemical Industry Institute of Toxicology. *90-day inhalation study of hydrogen chloride gas in B6C3F$_1$ mice, Sprague-Dawley rats and Fischer-344 rats.* ToxiGenics, Inc. for CIIT, Research Triangle Park NC, CIIT Docket No. 20915. Cited in the MAK documentation, Reference 22.

Table 3. NOAELs and LOAELs reported with inhalation exposure to low levels of nitric acid.

NOAEL (mg/m^3)	LOAEL (mg/m^3)	Subjects/ Species	Exposure time	Effect	Ref.
Human studies					
0.125	–	Asthmatic adolescents	40 minutes	No effect on lung function.	47
–	0.2	Healthy volunteers	2 hours including exercise	Effect on alveolar macrophages (higher phagocytosis and resistance to infection, lower superoxide anion production).	12
0.4	–	Healthy volunteers	2 hours	No effects on reported symptoms or lung function.	5
0.5	–	Healthy volunteers	4 hours	No effects on reported symptoms. No damage to respiratory passages measured with lung function tests, lavage and bronchial biopsy.	6
Animal studies					
–	0.05	Rat	40 weeks	Higher levels of stress-inducible heat shock protein 70 in lungs.	52, 73, 87
–	0.05	Rabbit	4 weeks	Reduced production of superoxide anions in alveolar macrophages.	68
0.05	0.15	Rabbit	4 weeks	Reduced bronchial reactivity *in vitro* on provocation with acetylcholine and histamine. Lower TNFα activity in alveolar macrophages.	68
–	0.25[a]	Rat	4 hrs/day 4 days	Lower superoxide anion production in isolated lung macrophages. Higher ability in lung lavage to inhibit elastase activity.	59
4.1 [b, c]	–	Sheep	4 hours	No bronchoconstriction (specific pulmonary flow resistance).	1
4.1 [b]	4.1 [c]	Sheep	4 hours	Hyperreactivity in respiratory passages (carbachol).	1

TNF = Tumor Necrosis Factor
[a] A 4-hour exposure to 1 mg/m^3 (same total dose) also increased ability to inhibit elastase activity.
[b] Not sensitized.
[c] Sensitized.

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Summary

Montelius J (ed). Swedish Criteria Group for Occupational Standards. *Scientific Basis for Swedish Occupational Standards.* XXX. Arbete och Hälsa 2010;44(5):1-124. University of Gothenburg, Sweden.

Critical review and evaluation of those scientific data which are relevant as a background for discussion of Swedish occupational exposure limits. This volume consists of the consensus reports given by the Criteria Group at the Swedish Work Environmental Authority from July, 2008 through June, 2009.

Key Words; Grain dust, Hydrochloric acid, Molybdenum, Molybdenum compounds, Nitric acid, Occupational exposure limit (OEL), Phosphoric acid, Risk assessment, Scientific basis, Styrene, Sulfuric acid, Toxicology.

Sammanfattning

Montelius J (ed). Kriteriegruppen för hygieniska gränsvärden. *Vetenskapligt underlag för hygieniska gränsvärden.* XXX. Arbete och Hälsa 2010;44;(5):1-124. Göteborgs Universitet.

Sammanställningar baserade på kritisk genomgång och värdering av de vetenskapliga fakta, vilka är relevanta som underlag för fastställande av hygieniskt gränsvärde. Volymen omfattar de underlag som avgivits från Kriteriegruppen för hygieniska gränsvärden under perioden juli 2008 – juni 2009.

Nyckelord; Fosforsyra, Hygieniskt gränsvärde, Molybden, Molybdenföreningar, Riskvärdering, Salpetersyra, Saltsyra, Spannmålsdamm, Styren, Svavelsyra, Toxikologi, Vetenskapligt underlag.

En svensk version av dessa vetenskapliga underlag finns publicerad i Arbete och Hälsa 2010;44(2):1-123.

APPENDIX

Consensus reports in this and previous volumes

Substance	Consensus date	Published in Arbete och Hälsa year;volume(No)	No. in series of Consensus Reports
Acetaldehyde	February 17, 1987	1987;39	VIII
Acetamide	December 11, 1991	1992;47	XIII
Acetic acid	June15, 1988	1988;32	IX
Acetone	October 20, 1987	1988;32	IX
Acetonitrile	September 12, 1989	1991;8	XI
Acrylamide	April 17, 1991	1992;6	XII
Acrylates	December 9, 1984	1985;32	VI
Acrylonitrile	April 28, 1987	1987;39	VIII
Aliphatic amines	August 25, 1982	1983;36	IV
Aliphatic hydrocarbons, C10-C15	June 1, 1983	1983;36	IV
Aliphatic monoketons	September 5, 1990	1992;6	XII
Allyl alcohol	September 9, 1986	1987;39	VIII
Allylamine	August 25, 1982	1983;36	IV
Allyl chloride	June 6, 1989	1989;32	X
Aluminum	April 21, 1982	1982;24	III
revised	September 14, 1994	1995;19	XVI
Aluminum trifluoride	September 15, 2004	2005;17	XXVI
p-Aminoazobenzene	February 29, 1980	1981;21	I
Ammonia	April 28, 1987	1987;39	VIII
revised	October 24, 2005	2006;11	XXVII
Ammonium fluoride	September 15, 2004	2005;17	XXVI
Amylacetate	March 23, 1983	1983;36	IV
revised	June 14, 2000	2000;22	XXI
Aniline	October 26, 1988	1989;32	X
Anthraquinone	November 26, 1987	1988;32	IX
Antimony + compounds	December 8, 1999	2000;22	XXI
Arsenic, inorganic	December 9, 1980	1982;9	II
revised	February 15, 1984	1984;44	V
Arsine	October 20, 1987	1988;32	IX
Asbestos	October 21, 1981	1982;24	III
Barium	June 16, 1987	1987;39	VIII
revised	January 26, 1994	1994;30	XV
Benzene	March 4, 1981	1982;9	II
revised	February 24, 1988	1988;32	IX
Benzoyl peroxide	February 13, 1985	1985;32	VI
Beryllium	April 25, 1984	1984;44	V
Borax	October 6, 1982	1983;36	IV
Boric acid	October 6, 1982	1983;36	IV

Boron Nitride	January 27, 1993	1993;37	XIV
Butadiene	October 23, 1985	1986;35	VII
1-Butanol	June 17, 1981	1982;24	III
Butanols	June 6, 1984	1984;44	V
Butyl acetate	June 6, 1984	1984;44	V
Butyl acetates	February 11, 1998	1998;25	XIX
Butylamine	August 25, 1982	1983;36	IV
Butyl glycol	October 6, 1982	1983;36	IV
γ-Butyrolactone	June 2, 2004	2005;7	XXV
Cadmium	January 18, 1980	1981;21	I
revised	February 15, 1984	1984;44	V
revised	May 13, 1992	1992;47	XIII
revised	February 5, 2003	2003;16	XXIV
Calcium fluorid	September 15, 2004	2005;17	XXVI
Calcium hydroxide	February 24, 1999	1999;26	XX
Calcium nitride	January 27, 1993	1993;37	XIV
Calcium oxide	February 24, 1999	1999;26	XX
Caprolactam	October 31, 1989	1991;8	XI
Carbon monoxide	December 9, 1981	1982;24	III
Cathecol	September 4, 1991	1992;47	XIII
Chlorine	December 9, 1980	1982;9	II
Chlorine dioxide	December 9, 1980	1982;9	II
Chlorobenzene	September 16, 1992	1993;37	XIV
revised	April 2, 2003	2003;16	XXIV
o-Chlorobenzylidene malononitrile	June 1, 1994	1994;30	XV
Chlorocresol	December 12, 1990	1992;6	XII
Chlorodifluoromethane	June 2, 1982	1982; 24	III
Chlorophenols	September 4, 1985	1986;35	VII
Chloroprene	April 16, 1986	1986;35	VII
Chromium	December 14, 1979	1981;21	I
revised	May 26, 1993	1993;37	XIV
revised	May 24, 2000	2000;22	XXI
Chromium trioxide	May 24, 2000	2000;22	XXI
Coal dust	September 9, 1986	1987;39	VIII
Cobalt	October 27, 1982	1983;36	IV
Cobalt and cobalt compounds	October 22, 2003	2005;7	XXV
Copper	October 21, 1981	1982;24	III
Cotton dust	February14, 1986	1986;35	VII
Creosote	October 26, 1988	1989;32	X
revised	December 5, 2007	2009;43(4)	XXIX
Cresols	February 11, 1998	1998;25	XIX
Cumene	June 2, 1982	1982;24	III
Cyanamid	September 30, 1998	1999;26	XX
Cyanoacrylates	March 5, 1997	1997;25	XVIII
Cycloalkanes, C5-C15	April 25, 1984	1984;44	V
Cyclohexanone	March 10, 1982	1982;24	III
revised	February 24, 1999	1999;26	XX
Cyclohexanone peroxide	February 13, 1985	1985;32	VI

Cyclohexylamine	February 7, 1990	1991;8	XI
Desflurane	May 27, 1998	1998;25	XIX
Diacetone alcohol	December 14, 1988	1989;32	X
Dichlorobenzenes	February 11, 1998	1998;25	XIX
1,2-Dibromo-3-chloropropane	May 30, 1979	1981;21	I
Dichlorodifluoromethane	June 2, 1982	1982;24	III
1,2-Dichloroethane	February 29, 1980	1981;21	I
Dichloromethane	February 29, 1980	1981;21	I
Dicumyl peroxide	February 13, 1985	1985;32	VI
Dicyclopentadiene	March 23, 1994	1994;30	XV
Diesel exhaust	December 4, 2002	2003;16	XXIV
Diethanolamine	September 4, 1991	1992;47	XIII
Diethylamine	August 25, 1982	1983;36	IV
2-Diethylaminoethanol	January 25, 1995	1995;19	XVI
Diethylene glycol	September 16, 1992	1993;37	XIV
Diethyleneglycol ethylether + acetate	December 11, 1996	1997;25	XVIII
Diethyleneglycol methylether + acetate	March 13, 1996	1996;25	XVII
Diethyleneglycol monobutylether	January 25, 1995	1995;19	XVI
Diethylenetriamine	August 25, 1982	1983;36	IV
revised	January 25, 1995	1995;19	XVI
Diisocyanates	April 8, 1981	1982;9	II
revised	April 27, 1988	1988;32	IX
revised	May 30, 2001	2001;20	XXII
Diisopropylamine	February 7, 1990	1991;8	XI
N,N-Dimethylacetamide	March 23, 1994	1994;30	XV
Dimethyl adipate	December 9, 1998	1999;26	XX
Dimethylamine	December 10, 1997	1998;25	XIX
N,N-Dimethylaniline	December 12, 1989	1991;8	XI
Dimethyldisulfide	September 9, 1986	1987;39	VIII
Dimethylether	September 14, 1994	1995;19	XVI
Dimethylethylamine	June 12, 1991	1992;6	XII
Dimethylformamide	March 23, 1983	1983;36	IV
Dimethyl glutarate	December 9, 1998	1999;26	XX
Dimethylhydrazine	January 27, 1993	1993;37	XIV
Dimethyl succinate	December 9, 1998	1999;26	XX
Dimethylsulfide	September 9, 1986	1987;39	VIII
Dimethylsulfoxide, DMSO	December 11, 1991	1992;47	XIII
Dioxane	August 25, 1982	1983;36	IV
revised	March 4, 1992	1992;47	XIII
Diphenylamine	January 25, 1995	1995;19	XVI
4,4'-Diphenylmethanediisocyanate (MDI)	April 8, 1981	1982;9	II
revised	May 30, 2001	2001;20	XXII
Dipropylene glycol	May 26, 1993	1993;37	XIV
Dipropyleneglycol monomethylether	December 12, 1990	1992;6	XII
Disulfiram	October 31, 1989	1991;8	XI
Enzymes, industrial	June 5, 1996	1996;25	XVII
Ethanol	May 30, 1990	1991;8	XI

Ethanolamine	September 4, 1991	1992;47	XIII
Ethylacetate	March 28, 1990	1991;8	XI
Ethylamine	August 25, 1982	1983;36	IV
Ethylamylketone	September 5, 1990	1992;6	XII
Ethylbenzene	December 16, 1986	1987;39	VIII
Ethylchloride	December 11, 1991	1992;47	XIII
Ethylene	December 11, 1996	1997;25	XVIII
Ethylene chloride	February 29, 1980	1981;21	I
Ethylene diamine	August 25, 1982	1983;36	IV
Ethylene glycol	October 21, 1981	1982;24	III
Ethylene glycol ethylether + acetate	February 6	2009;43(4)	XXIX
Ethylene glycol methylether + acetate	June 2, 1999	1999;26	XX
Ethyleneglycol monoisopropylether	November 16, 1994	1995;19	XVI
Ethyleneglycol monopropylether + acetate	September 15, 1993	1994;30	XV
Ethylene oxide	December 9, 1981	1982;24	III
Ethylenethiourea	September 27, 2000	2001;20	XXII
Ethylether	January 27, 1993	1993;37	XIV
Ethylglycol	October 6, 1982	1983;36	IV
Ferbam	September 12, 1989	1991;8	XI
Ferric dimethyldithiocarbamate	September 12, 1989	1991;8	XI
Flour dust	December 10, 1997	1998;25	XIX
Fluorides	September 15, 2004	2005;17	XXVI
Formaldehyde	June 30, 1979	1981;21	I
revised	August 25, 1982	1983;36	IV
Formamide	December 12, 1989	1991;8	XI
Formic acid	June 15, 1988	1988;32	IX
Furfural	April 25, 1984	1984;44	V
Furfuryl alcohol	February 13, 1985	1985;32	VI
Gallium + Gallium compounds	January 25, 1995	1995;19	XVI
Glutaraldehyde	September 30, 1998	1999;26	XX
Glycol ethers	October 6, 1982	1983;36	IV
Glyoxal	September 13, 1996	1996;25	XVII
Grain dust	December 14, 1988	1989;32	X
revised	February 4, 2009	2010;44(5)	XXX
Graphite	December 10, 1997	1998;25	XIX
Halothane	April 25, 1985	1985;32	VI
2-Heptanone	September 5, 1990	1992;6	XII
3-Heptanone	September 5, 1990	1992;6	XII
Hexachloroethane	September 15, 1993	1994;30	XV
Hexamethylenediisocyanate (HDI)	April 8, 1981	1982;9	II
revised	May 30, 2001	2001;20	XXII
Hexamethylenetetramine	August 25, 1982	1983;36	IV
n-Hexanal	March 29, 2006	2006;11	XXVII
n-Hexane	January 27, 1982	1982;24	III
2-Hexanone	September 5, 1990	1992;6	XII
Hexyleneglycol	November 17, 1993	1994;30	XV

Hydrazine	May 13, 1992	1992;47	XIII
Hydrochloric acid	June 3, 2009	2010;44(5)	XXX
Hydrogen bromide	February 11, 1998	1998;25	XIX
Hydrogen cyanide	February 7, 2001	2001;20	XXII
Hydrogen fluoride	April 25, 1984	1984;44	V
revised	September 15, 2004	2005;17	XXVI
Hydrogen peroxide	April 4, 1989	1989;32	X
Hydrogen sulfide	May 4, 1983	1983;36	IV
Hydroquinone	October 21, 1989	1991;8	XI
Indium	March 23, 1994	1994;30	XV
Industrial enzymes	June 5, 1996	1996;25	XVII
Isocyanic Acid (ICA)	December 5, 2001	2002;19	XXIII
Isophorone	February 20, 1991	1992;6	XII
Isopropanol	December 9, 1981	1982;24	III
Isopropylamine	February 7, 1990	1991;8	XI
Isopropylbenzene	June 2, 1982	1982;24	III
Lactates	March 29, 1995	1995;19	XVI
Lactate esters	June 2, 1999	1999;26	XX
Laughing gas	June 7, 2006	2006;11	XXVII
Lead, inorganic	February 29, 1980	1981;21	I
revised	September 5, 1990	1992;6	XII
revised	December 8, 2004	2005;17	XXVI
Lithium and lithium compounds	June 4, 2003	2003;16	XXIV
Lithium boron nitride	January 27, 1993	1993;37	XIV
Lithium nitride	January 27, 1993	1993;37	XIV
Maleic anhydride	September 12, 1989	1991;8	XI
Manganese	February 15, 1983	1983;36	IV
revised	April 17, 1991	1992;6	XII
revised	June 4, 1997	1997;25	XVIII
Man made mineral fibers	March 4, 1981	1982;9	II
revised	December 1, 1987	1988;32	IX
Mercury, inorganic	April 25, 1984	1984;44	V
Mesityl oxide	May 4, 1983	1983;36	IV
Metal stearates, some	September 15, 1993	1994;30	XV
Methacrylates	September 12, 1984	1985;32	VI
Methanol	April 25, 1985	1985;32	VI
Methyl acetate	March 28, 1990	1991;8	XI
Methylamine	August 25, 1982	1983;36	IV
Methylamyl alcohol	March 17, 1993	1993;37	XIV
Methyl bromide	April 27, 1988	1988;32	IX
Methyl chloride	March 4, 1992	1992;47	XIII
Methyl chloroform	March 4, 1981	1982;9	II
4,4´-methylene-bis-(2-chloroaniline)	February 4, 2004	2005;7	XXV
Methylene chloride	February 29, 1980	1981;21	I
4,4'-Methylene dianiline	June 16, 1987	1987;39	VIII
revised	October 3, 2001	2002;19	XXIII

Methyl ethyl ketone	February 13, 1985	1985;32	VI
Methyl ethyl ketone peroxide	February 13, 1985	1985;32	VI
Methyl formate	December 12, 1989	1991;8	XI
Methyl glycol	October 6, 1982	1983;36	IV
Methyl iodide	June 30, 1979	1981;21	I
Methylisoamylamine	September 5, 1990	1992;6	XII
Methylisoamylketone	February 6, 2002	2002;19	XXIII
Methylisocyanate (MIC)	December 5, 2001	2002;19	XXIII
Methyl mercaptane	September 9, 1986	1987;39	VIII
Methyl methacrylate	March 17, 1993	1993;37	XIV
Methyl pyrrolidone	June 16, 1987	1987;39	VIII
α-Methylstyrene	November 1, 2000	2001;20	XXII
Methyl-t-butyl ether	November 26, 1987	1988;32	IX
revised	September 30, 1998	1999;26	XX
Mixed solvents, neurotoxicity	April 25, 1985	1985;32	VI
MOCA	February 4, 2004	2005;7	XXV
Molybdenum	October 27, 1982	1983;36	IV
revised	Februari 4, 2009	2010;44(5)	XXX
Monochloroacetic acid	February 20, 1991	1992;6	XII
Monochlorobenzene	September 16, 1993	1993;37	XIV
Monomethylhydrazine	March 4, 1992	1992;47	XIII
Mononitrotoluene	February 20, 1991	1992;6	XII
Monoterpenes	February 17, 1987	1987;39	VIII
Morpholine	December 8, 1982	1983;36	IV
revised	June 5, 1996	1996;25	XVII
Naphthalene	May 27, 1998	1998;25	XIX
Natural crystallinic fibers, except asbestos	June 12, 1991	1992;6	XII
Nickel	April 21, 1982	1982;24	III
Nicotine	June 2, 2004	2005;7	XXV
Nitric acid	June 3, 2009	2010;44(5)	XXX
Nitric oxide	December 11, 1985	1986;35	VII
revised	June 13, 2007	2008;42(6)	XXVIII
Nitroethane	April 4, 1989	1989;32	X
Nitrogen dioxide	December 11, 1985	1986;35	VII
revised	September 12, 2007	2008;42(6)	XXVIII
Nitrogen oxides	December 11, 1985	1986;35	VII
Nitroglycerin	February 13, 1985	1985;32	VI
Nitroglycol	February 13, 1985	1985;32	VI
Nitromethane	January 6, 1989	1989;32	X
Nitropropane	October 28, 1986	1987;39	VIII
2-Nitropropane	March 29, 1995	1995;19	XVI
Nitroso compounds	December 12, 1990	1992;6	XII
Nitrosomorpholine	December 8, 1982	1983;36	IV
Nitrotoluene	February 20, 1991	1992;6	XII
Nitrous oxide	December 9, 1981	1982;24	III
revised	June 7, 2006	2006;11	XXVII
Oil mist	April 8, 1981	1982;9	II

Organic acid anhydrides, some	September 12, 1989	1991;8	XI
revised	June 4, 2008	2009;43(4)	XXIX
Oxalic acid	February 24, 1988	1988;32	IX
Ozone	April 28, 1987	1987;39	VIII
revised	February 7, 2007	2008;42(6)	XXVIII
Paper dust	February 7, 1990	1991;8	XI
Penicillins	November 23, 2005	2006;11	XXVII
Pentaerythritol	November 16, 1994	1995;19	XVI
1,1,1,2,2-Pentafluoroethane	February 24, 1999	1999;26	XX
Pentyl acetate	June 14, 2000	2000;22	XXI
Peroxides, organic	February 13, 1985	1985;32	VI
Phenol	February 13, 1985	1985;32	VI
Phosphoric acid	June 3, 2009	2010;44(5)	XXX
Phosphorous chlorides	September 30, 1998	1999;26	XX
Phosphorous oxides	February 11, 1998	1998;25	XIX
Phthalates	December 8, 1982	1983;36	IV
Phthalic anhydride	September 12, 1989	1991;8	XI
Piperazine	September 12, 1984	1985;32	VI
Plastic dusts	December 16, 1986	1987;39	VIII
Platinum	June 4, 1997	1997;25	XVIII
Polyaromatic hydrocarbons	February 15, 1984	1984;44	V
Polyisocyanates	April 27, 1988	1988;32	IX
Potassium aluminium fluoride	June 4, 1997	1997;25	XVIII
Potassium cyanide	February 7, 2001	2001;20	XXII
Potassium dichromate	May 24, 2000	2000;22	XXI
Potassium Fluoride	September 15, 2004	2005;17	XXVI
Potassium hydroxide	Marsh 15, 2000	2000;22	XXI
2-Propanol	December 9, 1981	1982;24	III
Propene	September 13, 1996	1996;25	XVII
Propionic acid	November 26, 1987	1988;32	IX
Propylacetate	September 14, 1994	1995;19	XVI
Propylene glycol	June 6, 1984	1984;44	V
Propylene glycol-1,2-dinitrate	May 4, 1983	1983;36	IV
Propylene glycol monomethylether	October 28, 1986	1987;39	VIII
Propylene oxide	June 11, 1986	1986;35	VII
Pyridine	May 13, 1992	1992;47	XIII
Quartz	March 13, 1996	1996;25	XVII
Resorcinol	September 4, 1991	1992;47	XIII
Selenium	December 11, 1985	1986;35	VII
revised	February 22, 1993	1993;37	XIV
Sevoflurane	May 27, 1998	1998;25	XIX
Silica	March 13, 1996	1996;25	XVII
Silver	October 28, 1986	1987;39	VIII
Sodium cyanide	February 7, 2001	2001;20	XXII
Sodium Fluoride	September 15, 2004	2005;17	XXVI

Sodium hydroxide	August 24, 2000	2000;22	XXI
Stearates, metallic, some	September 15, 1993	1994;30	XV
Stearates, non-metallic, some	November 17, 1993	1994;30	XV
Strontium	January 26, 1994	1994;30	XV
Styrene	February 29, 1980	1981;21	I
revised	October 31, 1989	1991;8	XI
revised	April 1, 2009	2010;44(5)	XXX
Sulfur dioxide	April 25, 1985	1985;32	VI
Sulfur fluorides	March 28, 1990	1991;8	XI
Sulfuric acid	June 3, 2009	2010;44(5)	XXX
Synthetic inorganic fibers	March 4, 1981	1982;9	II
revised	December 1, 1987	1988;32	IX
revised	December 3, 2003	2005;7	XXV
Synthetic organic and inorganic fibers	May 30, 1990	1991;8	XI
Talc dust	June 12, 1991	1992;6	XII
Terpenes, mono-	February 17, 1987	1987;39	VIII
Tetrabromoethane	May 30, 1990	1991;8	XI
Tetrachloroethane	June 4, 1997	1997;25	XVIII
Tetrachloroethylene	February 29, 1980	1981;21	I
1,1,1,2-Tetrafluoroethane	March 29, 1995	1995;19	XVI
Tetrahydrofuran	October 31, 1989	1991;8	XI
Tetranitromethane	April 4, 1989	1989;32	X
Thioglycolic acid	June 1, 1994	1994;30	XV
Thiourea	December 1, 1987	1988;32	IX
revised	June 2, 1999	1999;26	XX
Thiram	October 31, 1989	1991;8	XI
Thiurams, some	October 31, 1989	1991;8	XI
Tin and inorganic tin compounds	October 22, 2003	2005;7	XXV
Titanium dioxide	February 21, 1989	1989;32	X
Toluene	February 29, 1980	1981;21	I
revised	February 6, 2002	2002;19	XXIII
Toluene-2,4-diamine	November 1, 2000	2001;20	XXII
Toluene-2,6-diamine	November 1, 2000	2001;20	XXII
Toluene-2,4-diisocyanate	April 8, 1981	1982;9	II
revised	May 30, 2001	2001;20	XXII
Toluene-2,6-diisocyanate	April 8, 1981	1982;9	II
revised	May 30, 2001	2001;20	XXII
1,1,1-Trifluoroethane	February 24, 1999	1999;26	XX
Trichlorobenzene	September 16, 1993	1993;37	XIV
1,1,1-Trichloroethane	March 4, 1981	1982;9	II
Trichloroethylene	December 14, 1979	1981;21	I
Trichlorofluoromethane	June 2, 1982	1982;24	III
1,1,2-Trichloro-1,2,2-trifluoroethane	June 2, 1982	1982;24	III
Triethanolamine	August 25, 1982	1983;36	IV
revised	October 23, 2002	2003;16	XXIV
Triethylamine	December 5, 1984	1985;32	VI
Trimellitic anhydride	September 12, 1989	1991;8	XI
Trimethylolpropane	November 16, 1994	1995;19	XVI

Trinitrotoluene	April 17, 1991	1992;6	XII
Vanadium	March 15, 1983	1983;36	IV
Vinyl acetate	June 6, 1989	1989;32	X
Vinyl toluene	December 12, 1990	1992;6	XII
White spirit	December 16, 1986	1987;39	VIII
revised	November 13, 2006	2008;42(6)	XXVIII
Wood dust	June 17, 1981	1982;9	II
revised	June 25, 2000	2000;22	XXI
Xylene	February 29, 1980	1981;21	I
revised	September 14, 2005	2005;17	XXVI
Zinc	April 21, 1982	1982;24	III
Zinc chromate	May 24, 2000	2000;22	XXI
Zinc dimethyl dithiocarbamate	September 12, 1989	1991;8	XI
Ziram	September 12, 1989	1991;8	XI

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