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Scientific Basis for Swedish Occupational Standards xxv

Ed. Johan Montelius
Criteria Group for Occupational Standards
National Institute for Working Life
S-113 91 Stockholm, Sweden

Translation:
Frances Van Sant
(except for the consensus report on Cobalt and Cobalt Compounds which was originally written in English)

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Preface

The Criteria Group of the Swedish National Institute for Working Life (NIWL) has the task of gathering and evaluating data which can be used as a scientific basis for the proposal of occupational exposure limits given by the Swedish Work Environment Authority (SWEA). In most cases a scientific basis is written on request from the SWEA. The Criteria Group shall not propose a numerical occupational exposure limit value but, as far as possible, give a dose-response/dose-effect relationship and the critical effect of occupational exposure.

In searching of the literature several databases are used, such as RTECS, Toxline, Medline, Cancerlit, Nioshtic and Riskline. Also information in existing criteria documents is used, e.g. documents from WHO, EU, US NIOSH, the Dutch Expert Committee for Occupational Standards (DECOS) and the Nordic Expert Group (NEG). In some cases criteria documents are produced within the Criteria Group, often in collaboration with DECOS or US NIOSH.

Evaluations are made of all relevant published original papers found in the searches. In some cases information from handbooks and reports from e.g. US NIOSH and US EPA is used. A draft consensus report is written by the secretariat or by a scientist appointed by the secretariat. The author of the draft is indicated under Contents. A qualified evaluation is made of the information in the references. In some cases the information can be omitted if some criteria are not fulfilled. In some cases such information is included in the report but with a comment why the data are not included in the evaluation. After discussion in the Criteria Group the drafts are approved and accepted as a consensus report from the group. They are sent to the SWEA.

This is the 25th volume that is published and it contains consensus reports approved by the Criteria Group during the period July 2003 through June 2004. These and previously published consensus reports are listed in the Appendix (p 117).

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Johan Montelius
Secretary

The Criteria Group has the following membership (as of June, 2004)

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Christer Edling		Dept Environ Occup Medicine, University Hospital, Uppsala
Per Eriksson		Dept Environmental Toxicology, Uppsala University
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Kjell Larsson		Inst Environmental Medicine, Karolinska Institutet
Carola Lidén		Dept Occup Environ Health, Stockholm County Council
Johan Montelius	secretary	Dept for Work and Health, Natl Inst for Working Life
Gun Nise		Dept Occupational Medicine, Norrbacka, Stockholm
Göran Pettersson		Swedish Industrial Workers Union
Bengt Sjögren		Inst Environmental Medicine, Karolinska Institutet
Birgitta Pettersson	observer	Swedish Work Environment Authority
Kerstin Wahlberg	observer	Swedish Work Environment Authority
Marianne Walding	observer	Swedish Work Environment Authority
Olof Vesterberg		Natl Inst for Working Life

Contents

[1] Drafted by Birgitta Lindell, Department for Work and Health, National Institute for Working Life, Sweden.

[2] Drafted by Nicole Palmen, Arbodienst Limburg, Holland.

[3] Drafted by Peter Westerholm, Department for Work and Health, National Institute for Working Life, Sweden; Staffan Krantz, National Institute for Working Life, Sweden.

[4] Drafted by Ilona Silins, Institute of Environmental Medicine, Karolinska Institutet, Sweden.

[5] Drafted by Stefan Willers, Unit of Preventive Medicine, Heart and Lung Center and Dept of Occupational and Environmental Medicine, Lund University Hospital, Sweden.

[6] Drafted by Birgitta Lindell, Department for Work and Health, National Institute for Working Life, Sweden.

Consensus Report for Tin and Inorganic Tin Compounds

October 22, 2003

This Consensus Report is based primarily on a criteria document compiled jointly by the Nordic Expert Group and the Dutch Expert Committee (65).

Chemical and physical data

Name chemical formula	CAS No.	Molecular weight	Melting point (°C)	Boiling point (°C)	Solubility in water
Tin Sn	7440-31-5	118.7	231.9	2602	insoluble
Potassium stannate $K_2Sn(OH)_6$	12125-03-0	298.9	-	-	soluble
Sodium stannate $Na_2Sn(OH)_6$	12209-98-2	266.7	140	-	soluble
Tin(IV) bromide $SnBr_4$	7789-67-5	438.3	31	205	soluble
Tin(II) chloride $SnCl_2$	7772-99-8	189.6	247	623	soluble
Tin(IV) chloride $SnCl_4$	7646-78-8	260.5	-33	114	soluble
Tin(IV) chloride iodide $SnCl_2I_2$	13940-16-4	443.4	-	297	soluble
Tin(II) fluoride SnF_2	7783-47-3	156.7	213	850	soluble
Tin(II) iodide SnI_2	10294-70-9	372.5	320	714	somewhat soluble
Tin(IV) iodide SnI_4	7790-47-8	626.3	143	364.5	soluble
Tin(II) oxide SnO	21651-19-4	134.7	1080	-	insoluble
Tin(IV) oxide SnO_2	18282-10-5	150.7	1630	1900	insoluble
Tin(II) pyrophosphate $Sn_2P_2O_7$	15578-26-4	411.3	disintegrates at 400 °C		insoluble
Tin(II) sulfide SnS	12738-87-3	150.8	880	1210	insoluble
Tin(II) sulfate $SnSO_4$	7488-55-3	214.8	disintegrates at >378 °C (SO_2)	-	soluble

At room temperature tin is a shiny, silver-white metal (white tin) that develops a thin oxide layer on exposure to dry air or oxygen. Below 13.2 °C white tin slowly crumbles to a gray powder (gray tin). At 200 °C white tin is transformed to brittle tin. Tin reacts with strong acids and bases but is relatively resistant to neutral solutions. Tin occurs naturally in ten stable isotopes. Tin in compounds has an oxidation number of +II or +IV. The water solubility of tin compounds varies (25, 35, 65). Simple inorganic tin salts are hydrolyzed and form acids (2). $SnCl_4$ is hydrolyzed by water in a violent reaction producing hydrogen chloride and quite a bit of heat (25).

Occurrence, use

Tin is mined primarily as the mineral cassiterite (tin stone), SnO_2. Other ores containing tin include stannite (Cu_2FeSnS_4) and teallite ($PbZnSnS_2$) (25, 65). Metallic tin is obtained from tin ore by smelting. The metal is widely used as a corrosion-resistant plating on other metals (tin plating, tinning) such as sheet steel for "tin" cans for the food industry. Tin is also used in alloys ranging from solder to dental amalgam (2, 25, 65). Inorganic tin compounds are used in the production of ceramics, porcelain, enamel, drill glass, textiles (to fix dyes), ink and tooth-paste. $SnCl_2$ is widely used as a reducing agent in production of ceramics, glass and ink. $SnCl_4$ occurs as a thickener in organic syntheses, as a stabilizer in plastics and as a chemical intermediate in the production of other tin compounds. SnO_2 is used as an opacifier in ceramics and as a pigment. SnF_2 is used in dentistry (6, 65).

Uptake, biotransformation, excretion

In general, uptake of inorganic tin via the digestive tract is low. Data indicate, however, that uptake can be dose-dependent and also dependent on the anion. One study with human subjects reports that when 0.11 mg Sn/day was ingested in food about 50% of the dose was absorbed, whereas uptake was only 3% in subjects on a diet containing a further 50 mg Sn/day (administered as $SnCl_2$) (65). There are no data on uptake via lungs or skin.

Human data and data from animal experiments indicate that very little inorganic tin passes the blood-brain barrier. Inorganic tin accumulates primarily in bone, but some accumulation has also been shown in human lungs, liver, kidneys, adrenals, lymph nodes and testes. Some data also indicate that tin has a higher affinity for thymus than for other organs. The biological half time for Sn(II) and Sn(IV) in the bones of rats is reported to be 20 to 100 days, and the half time for Sn(II) in rat liver and kidney 10 to 20 days (65). Differences between Sn(II) and Sn(IV) in their relative affinity to kidney and liver suggest that tin is not rapidly oxidized or reduced during absorption and systemic transport. Animal data on differences between $SnCl_2$ and $SnCl_4$ with regard to effects on the immune system also indicate valence stability *in vivo* (65).

Absorbed tin is excreted primarily via the kidneys. In a study with rats, it is reported that 35% of an injected (i.v.) dose of Sn(II) citrate and 40% of a dose of Sn(IV) citrate were excreted in urine – most of it within 10 hours. Excretion in feces amounted to 12% of the Sn(II) but only 3% of the Sn(IV), which indicates that excretion in bile is more important for Sn(II) compounds than for Sn(IV) compounds (23, 65).

Toxic effects

Human data

There are some reports that tin may be able to cause metal fume fever, but no primary data supporting this assertion have been published (1, 5, 24, 39, 47, 60, 64).

An accumulation of tin in the lungs, visible on x-rays but with no effect on lung function (stannosis, a form of pneumoconiosis), has been reported in workers exposed to dust/fumes of SnO_2 for 3 years or longer in tin foundries and scrap metal recycling plants and around tin plating. These case reports usually contain no information on exposure levels (65). In a study of 215 workers in a tin foundry, x-ray changes indicating stannosis were observed in 121 of them. There were no indications of fibrosis or clinically significant emphysema. Nor did any of the workers have clinical symptoms that could be ascribed to the occurrence of stannosis, and lung function tests (FEV and airway resistance) showed no indication of reduced lung function (50, 51, 52). These authors also report mortality among 607 men who had been employed in the tin foundry for at least 3 years in the 1921 – 1955 period. The population had lower mortality than predicted (51). Dust levels up to 2.22 mg Sn/m^3 were reported in the foundry, but no details (analysis methods etc.) are given (51). Another study (Hlebnikova 1957, reviewed in Reference 26) reports that workers developed stannosis after 6 to 8 years of work at the smelting ovens. The workers were exposed to aerosols formed during the smelting process and consisting primarily of SnO_2 (<3% total silicon dioxide). Total dust concentrations in air ranged from 3 to 70 mg/m^3. The dust concentration was reduced to 10 mg/m^3, and no new cases were reported in the following ten years.

A smaller study (questionnaire) reports elevated prevalence of wheezing, chest pains, coughing and shortness of breath with physical exertion in workers who were exposed for about 7 hours/day to fumes consisting mainly of $SnCl_4$ and the hydrogen chloride formed when the $SnCl_4$ combined with moisture in the heated air. According to the authors, the effects were probably due primarily to the hydrogen chloride exposure, although smoking may have contributed to the elevated frequencies of coughing and shortness of breath. Up to 0.18 mg $SnCl_4$/m^3 and up to 5 ppm hydrogen chloride were measured in the air. However, the air concentrations were not monitored until after radical measures had been taken to improve the work environment, and thus do not reflect the exposure situation at the time of the questionnaire (34).

A Belgian case-control study (n = 272) reports a significant increase in risk for chronic kidney failure (odds ratio 3.72; 95% CI 1.22 – 11.3) in persons with occupational exposure to tin (42).

Ingested tin can have an irritating effect on the digestive tract. There are many reports of acute poisoning after intake of canned fruit or fruit juice. The most common symptoms are nausea, vomiting, diarrhea and stomach cramps (65). In one study with volunteers (3), nausea and diarrhea were reported after a single glass of orange juice containing 1370 mg Sn/liter (intake of about 330 mg Sn, or about 4.4 – 6.7 mg Sn/kg b.w.). No effects were observed after intake of a glass of juice containing 540 mg Sn/l (about 130 mg Sn; 1.7 – 2.7 mg Sn/kg b.w.). In another study, however, nausea, cramps and loose stools were reported after a single dose of 100 mg Sn (as $SnCl_2$) in Coca Cola. According to the authors, the concentration of Sn in the test solution was about the same as that which produced the symptoms described in Reference 3 (56).

Daily intake of fruit juice containing 50 mg Sn (as $SnCl_2$) for 20 days (about 0.7 mg Sn/kg b.w./day) was found to increase excretion of zinc and selenium in feces and reduce retention of zinc and excretion of zinc in urine. No significant effects on excretion of calcium, copper, iron, manganese or magnesium were observed (20, 28, 29). Inhibited zinc absorption, measured as retention of radioactively labeled zinc in the body after 7 to 10 days, is reported in another work in which $SnCl_2$ (36 mg Sn) in a $ZnCl_2$ solution/diet containing zinc was given to subjects on a single occasion (63). In the study in which volunteers were given zinc in Coca Cola containing up to 100 mg Sn as $SnCl_2$, however, there was no clear reduction in uptake of zinc, measured as reduction of zinc in plasma after 1 to 4 hours (56).

Positive reactions in patch tests, regarded as expressions of an allergic reaction, have been reported for metallic Sn or 1% or 2% $SnCl_2$ in petrolatum. $SnCl_2$, 5% or 10% in petrolatum, is reported to be irritating to skin (13, 16, 38, 49). Contact eczema was reported in one worker who had been exposed to dust from an alloy containing 43% tin. The patient also had a positive result in a patch test with 1% $SnCl_2$ in petrolatum, and the case was judged to be an occupation-related allergic contact dermatitis to tin (40). Considering that tin is a widely occurring substance and that only a single case of allergic contact eczema has been definitely attributed to tin exposure, it must be concluded that tin and tin compounds very seldom cause contact allergy.

Animal data

When rats were given a single intratracheal instillation of 50 mg tin dust from a tin smelter (in saline), an accumulation of dust was observed in the lungs, but there were no indications of changes in connective tissue during the year following the exposure (50). Greater susceptibility to lung infections was noted in a study in which mice were given a single intratracheal instillation of 0.01 or 0.1 mg $SnCl_2$ in saline (equivalent to about 0.25 or 2.5 mg Sn/kg b.w.) and then exposed to a bacterial aerosol. Reported increases in mortality were 36% and 87%

respectively (22). An older study reports that temporary irritation of eyes and noses were the only observed effects on guinea pigs exposed by inhalation to 3000 mg/m^3 $SnCl_4$, 10 minutes per day "for months" (45).

The LD_{50} (24 hours) for oral administration to laboratory rodents is reported in one study to be 146 – 396 mg Sn/kg b.w. for $NaSn_2F_5$, and 1197 – 1678 mg Sn/kg b.w. for $SnCl_2$. With intraperitoneal injection, the LD_{50} for rats (24 hours) was 43 – 50 mg Sn/kg b.w. for the former substance and 136 mg Sn/kg b.w. for the latter. The toxic picture, characterized by a soporific effect on the central nervous system and ataxia, was attributed to the Sn and (in the latter case) F. Both substances resulted in pathological changes in kidneys (tubular necroses, regeneration) (11).

The effects of inorganic tin compounds given in oral doses vary with such factors as the compound's solubility in water (14). In one study, rats were given feed containing 0.03, 0.1, 0.3 or 1% of various tin salts and tin oxides for 4 weeks ($SnCl_2$, Sn orthophosphate, Sn sulfate, SnS, SnO_2, Sn oxalate, Sn tartrate, Sn oleate) or 13 weeks ($SnCl_2$, SnO). No noteworthy effects (growth, hematology, histology, organ weights etc.) were reported at any dose level for SnS, SnO_2, SnO or Sn oleate. The 4-week exposures resulted in inhibited growth, histological changes in livers (possibly an effect of partial starvation) and indications of anemia in rats given 0.3 or 1% $SnCl_2$, Sn orthophosphate, Sn sulfate, Sn oxalate or Sn tartrate. Similar effects were observed in the 13-week experiment with $SnCl_2$. However, mortality at the highest dose level was high in this experiment, and for this dose group the exposure was stopped before the scheduled time. The NOEL for the 'active' tin salts in this study was estimated by the authors to be 0.1%, a level said to yield an intake of 22 – 33 mg Sn/kg b.w./day in a 90-day study. It was suggested that with a diet containing less iron and copper the NOEL might be lower (14, 65).

In another study in which $SnCl_2$ was given to rats in feed for 4 weeks, observed effects included lower body weights and reduction of hemoglobin at an average intake of about 30 mg Sn/kg b.w./day (27). In a 30-day study in which rats were given oral doses of 20, 100 or 175 mg $NaSn_2F_5$/kg b.w./day (about 13.4, 67, or 117 mg Sn/kg b.w./day), observed effects included dose-related growth inhibition, degenerative changes in proximal renal tubuli (15 – 20% of the high-dose group) and significant reduction of hemoglobin levels (males in the two highest dose groups, day 15). According to the authors, effects on animals in the lowest dose group were minimal (significantly lower body weights and serum glucose on one occasion) (10).

No noteworthy histopathological observations of non-neoplastic nature are reported in a long-term study (105 weeks) in which rats and mice were given 0.1 or 0.2% $SnCl_2$ in feed. The intake in the low-dose group can be calculated to be 20 – 50 mg Sn/kg b.w./day for the rats and 80 – 180 mg Sn/kg b.w./day for the mice (41). In older literature, however, effects of long-term oral administration of very low doses of tin have been reported to occur. In a study with rats, lifetime exposure to $SnCl_2$ in drinking water was reported to result in significant ($p<0.001$) increase in fatty degeneration of the liver (both sexes) at an intake equivalent to about 0.4 mg Sn/kg b.w./day (54). For moderate/severe fatty degeneration,

however, the difference between the control group and the treated group was less significant ($p<0.05$), and a larger proportion of controls than treated animals were reported to have 'degeneration and necrosis' in the liver. A somewhat elevated occurrence of tubular vacuolization in kidneys ($p<0.05$) was also reported in both sexes. Further, significantly elevated serum glucose levels and somewhat shortened life spans were noted in females, and in males somewhat lower weight gain (54). However, this study was not made according to modern praxis and the observations can not be interpreted with the documentation provided. Lifelong exposure to $SnCl_2$ in drinking water at a dose level of 0.4 mg Sn/kg b.w./day resulted in no noteworthy effects on mice (53).

Effects on iron, copper and zinc status have also been reported in some studies in which low doses of $SnCl_2$ were given orally to experimental animals (Table 1). In a study (46) in which rats were given feed containing 0.5 – 226 mg Sn/kg (as $SnCl_2$) for 28 days, it is reported that tissue and plasma concentrations of iron, copper and zinc were somewhat reduced at the dose level 1 mg Sn/kg b.w./day (10 mg Sn/kg feed). Increasing tin content in feed was associated with a dose-dependent reduction of iron in plasma (significantly lower than controls only at the higher dose levels) and a generally dose-dependent reduction of iron in kidneys, spleen and tibias. There was also a generally dose-dependent reduction of copper concentration in plasma, liver, kidneys, spleen and tibias, and of zinc concentration in plasma, kidneys and tibias. The hemoglobin concentration in blood also decreased with increasing doses of tin, but was lower than controls only at the highest dose. A percentual reduction of transferrin saturation with increasing tin dose was also observed (significantly lower than in controls only at higher doses). The statistical assessment was made using analysis of variance and test for linear trend.

Reduced calcium in bones, inhibited collagen synthesis and lowered enzyme activity, especially in bones, have also been demonstrated in studies in which rodents were given low oral doses of $SnCl_2$ (Table 1). In one study, rats were given oral doses of 0.3, 1 or 3 mg Sn/kg b.w. (as $SnCl_2$ in solution) twice daily for 90 days: there was a non-significant reduction of calcium in femurs at the lowest dose level (0.6 mg Sn/kg b.w./day), and at the higher dose levels (2 and 6 mg Sn/kg b.w./day) significant reductions of calcium in femurs as well as significant reductions in enzyme activity. At the highest level significant reductions in serum calcium and relative femur weight were also observed (66). These authors also report reduced calcium content in bones of rats after 28 days, and reduced enzyme activity in bones after only 3 days of oral administration of 1 mg Sn/kg b.w., twice a day for up to 28 days. Inhibited collagen synthesis in femurs was also reported (67, 68).

Tin cations have been shown to affect several different enzyme systems in experimental animals. This may interfere with the oxidative function in the cells and affect the detoxification of chemical substances (65). Reduced activity of the enzyme δ-aminolevulinic acid dehydratase (ALAD) was observed in the blood of rats after administration (oral, intraperitoneal, subcutaneous) of 2 doses of $SnCl_2$

(total 4 mg Sn/kg b.w.). Other studies report that ALAD is not inhibited by $SnCl_4$ (65). Dose-dependent induction of hemoxygenase in kidneys and livers of rats was reported after a single subcutaneous injection of $SnCl_2$ (3 – 30 mg Sn/kg b.w.). Significant inhibition of cytochrome P450-dependent liver enzymes and reduced levels of cytochrome P450 in liver microsomes were observed in mice after a single intravenous injection of 0.2 mg $SnCl_2$ /kg b.w. (0.1 mg Sn/kg b.w.) (7).

Patch tests on intact rabbit skin using 1% $SnCl_2$ or 0.25% SnF_2 in water produced no indications of skin irritation (57). The highest concentrations of $SnCl_2$ or $SnCl_4$ in alcohol that were non-irritating with a 1-minute application were 5% for rat skin, and 3% ($SnCl_2$) and 0.05% ($SnCl_4$) for oral mucosa (33). Neither substance caused sensitization when tested on rats (33).

Mutagenicity, genotoxicity

Tin compounds have been tested in several short-term *in vitro* tests, with contradictory results. $SnCl_2$ was reported to be negative in mutagenicity tests with *E.coli* WP2 and several strains of *Salmonella typhimurium*. SnF_2 was tested on the same *Salmonella* strains, and with metabolic activation showed a weak mutagenic effect on TA100 (19, 48). No DNA damage was indicated in two studies reporting tests of $SnCl_2$, $SnCl_4$ and $SnSO_4$ by the rec-assay system with *Bacillus subtilis* and of $SnCl_4$ by the SOS chromotest with *E.coli*, but $SnCl_2$ and $SnCl_4$ showed high toxicity in the rec-assay (21, 32). However, DNA damage has been reported in other *in vitro* studies in which $SnCl_2$ was tested on strains of *E.coli,* and also reduced survival of *E.coli* strains deficient in DNA repair (4, 44, 55). Experiments with *E.coli* have shown that one mechanism behind $SnCl_2$-induced damage (genotoxicity, cell death) may be production of reactive oxygen species (12). Dose-dependent increase of DNA damage was also observed when $SnCl_2$ was tested *in vitro* on mammalian cells and human white blood cells (36, 37). $SnCl_4$, however, did not cause DNA damage (36, 37), and in one of the experiments it was shown that the tin(IV) compound was not taken up in the cells (36). In other *in vitro* studies with human lymphocytes and $SnCl_4$, however, significant increases of chromosome aberrations, micronuclei and sister chromatid exchanges have been reported (17, 18, 59).

There are few *in vivo* studies. $SnCl_2$ was reported to be non-genotoxic in the *Drosophila* wing spot test (62). In another mutation test with *Drosophila*, the Basc test, it was concluded that SnF_2 was not mutagenic (19). SnF_2 was also negative in tests for micronuclei (bone marrow cells). In these tests the $SnCl_2$ was given to mice in two intraperitoneal injections (2 x 9.8, 2 x 19.6 or 2 x 39.5 mg/kg b.w.) (19).

Carcinogenicity

In a cancer study (41) feed containing 1000 or 2000 mg/kg $SnCl_2$ was given to rats and mice of both sexes for 105 weeks. A significantly elevated incidence of

C-cell adenomas in thyroid was noted in male rats in the low-dose group (controls 2/50; low-dose group 9/49; high-dose group 5/50) and the incidence of male rats with C-cell adenomas/carcinomas indicated a positive trend and significantly higher proportions in both dose groups (controls 2/50; low-dose group 13/49; high-dose group 8/50). However, the elevated incidence of C-cell tumors was not accompanied by an increase of C-cell hyperplasias. A significant positive trend for lung adenoma was also found in the male rats (controls 0/50; low-dose group 0/50; high-dose group 3/50). In the female mice there was a significant trend for hepatic adenomas/carcinomas (controls 3/49; low-dose group 4/49; high-dose group 8/49) and for a type of malignant lymphoma (controls 0/50; low-dose group 0/49; high-dose group 4/49). A comparison with historic controls (mice and rats) from the laboratory indicates that the tumor incidence was significantly elevated only for male rats in the low-dose group (C-cell tumors in thyroid). The intake in this group was calculated to be about 20 – 40 mg Sn/kg b.w./day. In the judgment of the authors, however, the increased incidence of thyroid tumors in this group could not be definitely related to the exposure, and they concluded that $SnCl_2$ was not carcinogenic to rats or mice.

No significant increase in the frequency of lung tumors was reported in a study in which mice were given three intraperitoneal injections of $SnCl_2$ per week (total 24 injections) and killed 30 weeks later. The total doses were 240 – 1200 mg/kg b.w. (150 – 750 mg Sn/kg b.w.) (58). Nor have long-term studies in which mice and rats were given small amounts of $SnCl_2$ in drinking water (amounting to about 0.4 mg Sn/kg b.w./day) yielded evidence that tin is carcinogenic (65). No evidence that tin is carcinogenic has been reported in several studies made with implantation or injection of metallic tin in laboratory rodents. Abnormal growth of glial tissue, however, was noted in a study in which metallic tin was implanted inside the skulls of mice (65).

No cancer studies of persons exposed only to tin were found. An elevated risk of lung cancer has been reported for miners in tin mines, but exposure to other substances such as radon, arsenic and tobacco were considered to be contributing factors (65).

Effects on reproduction

There are little data. Feed containing 125 – 500 mg Sn/kg as $NaSn_2F_5$ or $NaSn_2Cl_5$, or 156 – 625 mg Sn/kg as SnF_2, was given to rats during gestation. More resorptions were observed in a few of the mothers, nearly all of whom had been treated with $NaSn_2F_5$. The effect was not clearly dose-related and was considered to have no toxicological significance (61).

Dose-effect / dose-response relationships

Very few reliable measurements of air concentrations of inorganic tin compounds in work environments have been published, and it is therefore difficult to establish any direct dose-response or dose-effect relationships. An accumulation of tin in

the lungs, visible on x-rays but with no discernible effect on lung function (stannosis) has been observed in workers exposed to dust/smoke of SnO_2 for three years or more. One study reports dust concentrations up to 2.22 mg Sn/m^3 in a tin smelter where x-ray changes indicating stannosis were seen in 121 of 215 examined workers (50, 51, 52). No indications of fibrosis or clinically significant emphysema were observed. Nor did any of the workers have clinical symptoms that could be attributed to the occurrence of stannosis, and no indication of reduced lung function was found in lung function tests. In another study (Hlebnikova 1957, reviewed in Reference 26) it is reported that workers exposed to aerosols consisting primarily of SnO_2 developed stannosis after 6 – 8 years of employment. The total dust concentration in the air ranged from 3 to 70 mg/m^3. The dust level was reduced to 10 mg/m^3 and it is reported that no new cases were observed in the ensuing ten years.

A dose-dependent increase in sensitivity to respiratory infection was reported in mice given a single intratracheal injection of 0.01 or 0.1 mg $SnCl_2$ in saline (calculated to be about 0.25 or 2.5 mg Sn/kg b.w.) and then exposed to a bacterial aerosol (22). Equivalent air concentrations calculated from this would be about 1.8 or 18 mg Sn/m^3 (assuming inhalation of 10 m^3 during an 8-hour work day, 100% uptake and a body weight of about 70 kg).

Intake of $SnCl_2$ in fruit juice daily for 20 days had effects on excretion of zinc and selenium in volunteers, at a dose level of 0.7 mg Sn/kg b.w./day (20, 28, 29). Inhibition of zinc absorption was reported in another work in which subjects were given single oral doses of 36 mg Sn as $SnCl_2$, or about 0.5 mg Sn/kg b.w. (63). Dose-effect relationships observed in experimental animals given tin compounds orally are summarized in Table 1. Reduced calcium content in bones has been reported with administration of $SnCl_2$ at a dose level of 0.6 mg Sn/kg b.w./day, and lower levels of iron, copper and zinc in plasma and tissues at a dose level of 1 mg Sn/kg b.w./day (46, 66).

Conclusions

There are no data from which to derive a critical effect of occupational exposure to tin and inorganic tin compounds. Accumulation of tin in the lungs has been shown to occur with occupational exposure to SnO_2, but there are no reports of evidence that this affects lung function or development of fibrosis. Inorganic tin salts can form acids on contact with water, and in this form can be irritating and even corrosive to air passages, eyes and skin.

Table 1. Exposure-effect relationships observed in laboratory animals after oral administration of inorganic tin compounds.

Exposure	Substance	Species	Effects	Ref.
1.4 mg Sn/kg feed, 28 days (0.14 mg Sn/kg bw/day)	$SnCl_2$	Rat	Minimal or no effects on concentrations of iron, copper and zinc in tissues and plasma	46
5 ppm in drinking water, lifelong (0.4 mg Sn/kg bw/day)	$SnCl_2$	Mouse	Tin had no toxic effects	53
0.3 mg Sn/kg bw, twice a day, 90 days	$SnCl_2$	Rat	Non-significant reduction of calcium content in femurs	66
10 mg Sn/kg feed, 28 days (1 mg Sn/kg bw/day)	$SnCl_2$	Rat	Reduced iron content in kidneys, reduced copper in plasma, liver, kidneys, spleen and tibias, reduced zinc in kidneys and tibias	46
1 mg Sn/kg bw, twice a day up to 28 days	$SnCl_2$	Rat	Reduced calcium in femurs, lower activity of acid and alkaline phosphatases in femurs, inhibited collagen synthesis in femurs	67, 68
1 mg Sn/kg bw, twice a day, 90 days	$SnCl_2$	Rat	Reduced calcium content in femurs, reduced activity of succinate dehydrogenase in liver and acidic phosphatases in femurs	66
2 mg Sn/kg bw, 2 doses 48 h apart	$SnCl_2$	Rat	Reduced ALAD activity in blood	65
2 mg Sn/kg bw/day, 5 days	$SnCl_2$	Rabbit	No effects on heme biosynthesis, reduced iron content in kidneys, reduced copper content in kidneys and liver	69, 70
2 mg Sn/kg bw/day 1 month	$SnCl_2$	Rabbit	Higher iron concentration in liver and kidneys, reduced copper content in bone marrow, reduced zinc content in bone marrow, increased zinc content in blood	69
3 mg Sn/kg bw twice a day 90 days	$SnCl_2$	Rat	Reduced relative femur weight and calcium content, reduced calcium in serum; reduced activity of succinate dehydrogenase in liver; acidic phosphatases in femur, LDH and alkaline phosphates in serum	66
10 mg Sn/kg bw/day 4 months	$SnCl_2$	Rabbit	Temporary hemolytic anemia, temporary rise of iron content in serum, increased total iron-binding capacity	9
100 mg Sn/kg feed, 27 days (11 mg Sn/kg bw/day)	$SnCl_2$	Rat	Reduced calcium and zinc content in tibias	30, 31
100 mg Sn/kg feed 4 weeks	$SnCl_2$	Rat	Reduced copper content in in duodenum, liver, kidneys and femurs, reduced zinc content in kidneys and femurs	65

Table 1. Continued.

Exposure	Substance	Species	Effects	Ref.
13.4 mg Sn/kg bw/day, 30 days	$NaSn_2F_5$	Rat	Minimal effects on body weight and serum glucose	10
0.1% in feed, 4 or 13 weeks (22-33 mg Sn/kg bw/day)	$SnCl_2$, Sn-o-phosphate, Sn sulfate, Sn oxalate, Sn tartrate	Rat	NOEL	14
260 mg Sn/kg feed, 4 weeks (29 mg Sn/kg bw/day)	$SnCl_2$	Rat	Reduced hemoglobin, reduced body weights, changes in intestines	27
0.1% in feed, 105 weeks (20-50 mg Sn/kg bw/day)	$SnCl_2$	Rat	Significantly higher incidence of thyroid tumors in males. Substance judged to be non-carcinogenic	41
300 mg Sn/l drinking water + 52 mg Sn/kg feed, 4 weeks	$SnCl_2$	Rat	Reduced compression resistance in femurs	43
67 mg Sn/kg bw/day, 30 days	$NaSn_2F_5$	Rat	Retarded growth, lower serum glucose, lower hemoglobin levels	10
0.3% in feed 4 or 13 weeks (70-100 mg Sn/kg bw/day)	Sn sulfate Sn tartrate $SnCl_2$ Sn-o-phosphate, Sn oxalate	Rat	Inhibited growth, indications of anemia Inhibited growth, indications of anemia, histological changes in liver	14
100 mg Sn/kg bw single dose	$SnCl_2$	Rabbit	Disturbed heme synthesis	8
117 mg Sn/kg bw/day, 30 days	$NaSn_2F_5$	Rat	Inhibited growth, lower serum glucose, lower hemoglobin levels, degenerative changes in proximal renal tubuli	10
0.1% in feed, 105 weeks (80-180 mg Sn/kg bw/day)	$SnCl_2$	Mouse	The substance was judged to be non-carcinogenic	41
0.1-0.8% in feed 13 weeks (males: 163-310 mg Sn/kg bw/day females: 153-340 mg Sn/kg bw/day	$SnCl_2$	Rat	Both sexes: Slight anemia, elevated relative kidney and liver weights, irritation of digestive tract, various degrees of pancreas atrophy Males: Slight growth inhibition, minor histological changes in livers	15

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Consensus Report for Cobalt and Cobalt Compounds

October 22, 2003

This report is an update of the Consensus Report published in 1983 (105) and is based on the criteria document "Cobalt and cobalt compounds" (83).

Chemical and physical data. Occurrence.

Cobalt

CAS No.:	7440-48-4
Formula	Co
Molecular weight:	58.93
Boiling point:	3100 °C (IARC: 2870 °C)
Melting point:	1493 °C (IARC: 1495 °C)

The average concentration of cobalt (Co) in the earth's crust is 20 μg/g but higher concentrations are found in nickel and copper ore deposits from which about 25,000 tons of Co metal are produced annually (65). Co has one naturally occurring isotope, ^{59}Co, and has magnetic properties. It can form alloys and is not corroded by air or water at ordinary temperature. Co is resistant to alkali but soluble in acids (38, 49, 71, 103, 118). The main oxidation states are +II and +III. Most commercially used Co compounds are water soluble bivalent salts (see Table 1).

The human body contains 1000 to 2000 μg of Co; most of it is found in liver (vitamin B12), kidney, heart and spleen, and low concentrations in serum, brain and pancreas (26, 65, 71).

The daily Co intake for the general population ranges between 1.7-100 μg; the diet being the main source. Environmental airborne Co concentrations are usually around 1 ng/m^3 but in heavily industrialised cities concentrations up to 10 ng/m^3 have been reported. Co concentrations in drinking water vary between 0.1-5μg/l. Tobacco is an insignificant Co source (38).

Table 1. Identity and solubility of various Co compounds. Data from ref. (40).

Compound name	Formula	Molecular weight	CAS no.	Solubility in water[1)]
Cobalt	Co	58.9	7440-48-4	insoluble
Cobalt(II) oxide	CoO	74.9	1307-96-6	3.13 mg/l
Cobalt(II,III) oxide	Co_3O_4	240.8	1308-06-1	insoluble
Cobalt(II) chloride	$CoCl_2$	129.8	7646-79-9	529 g/l (20ºC)
Cobalt(II) chloride hexahydrate	$CoCl_2$ x $6H_2O$	237.9	7791-13-1	767 g/l (0ºC)
Cobalt(II) sulphate	$CoSO_4$	155.0	10124-43-3	393 g/l (25ºC)
Cobalt(II) sulphate heptahydrate	$CoSO_4$ x $7H_2O$	281.1	10026-24-1	604 g/l (3ºC)
Cobalt aluminate blue	CoO,Al_2O_3		1333-88-6	insoluble
Stellite*	Co(48-58%), Cr,Ni,W alloy		12638-07-2	
Vitallium*	Co(56-68%), Cr,Mo alloy		12629-02-6	
Hard metal	Co(10-25%), WC mixture			

*Trade mark

The most important use of metallic Co is in alloys with other metals (e.g. chromium, nickel, copper, aluminium, beryllium and molybdenum). Alloys that are important regarding occupational exposure are stellite and vitallium (38). Cobalt is applied in the production of super alloys, permanent magnets, dental and surgical implants; but hard-metals (cemented carbides) are the most important (65). Hard-metals are produced using a powder metallurgy process (sintering) in which tungsten carbide particles and Co metal are mixed, heated in hydrogen atmosphere, pressed, shaped, sintered and grinded. Co acts as a binder for tungsten carbide (57). Co has also been used in certain polishing disks of microdiamonds cemented into ultrafine Co metal powder (22, 65, 109).

Co salts and Co oxides are used as catalysts in organic reactions or as drying agents in paints, lacquers, varnishes and printing-inks. Co oxides, Co-Zn silicate and spinels are used as pigments in glass, enamels, ceramic and porcelain products (24).

The main route of occupational exposure is the respiratory tract (dusts, fumes or mists containing Co) although skin contact is important (38, 64, 91). Occupational exposures mainly occur in hard-metal production, processing and use, during the production of Co powder, in the use of Co-containing pigments and driers and during regeneration of spent catalysts (38).

In the following overview of Co exposures, only studies using personal air sampling will be taken into account. Airborne Co exposures are highly dependent on the type of industry and the stage of the production process (see Table 2). In general, the highest exposure levels are found in the hard metal industry during handling of powders and pressing (51). Concentrations of Co in air during wet

Table 2. Occupational exposure to cobalt in various types of industries and at different production stages as measured by personal air sampling. The values are rounded off.

type of industry	n	process	mean (μg Co/m^3)	lowest	highest	Ref.
hard metal		mixing	459	7	6390	51
		pressing	33	48	2910	
		grinding	45	1	482	
cobalt refinery	82	no distinction	570* (> 50: 70%) (> 500: 25%)	2	7700	104
diamond/cobalt	16	mixing room		9	2860	31
saw production	7	oven room		6	51	
diamond polishing		polishing	5.3-15	0.2	43	78
dental protheses	3	melting bay	4			58
production	3	refinishing bay	10	3	50	
dental protheses production	79		100 (2.5%) 25-100 (13.9%) <25 (83.6%)			45
dental technicians	8	not described		<detection	1600	95
pottery painting	19	plate painting	33	22	80	15
pottery painting	19	plate painting	> 50 (20%)**	68	8610	88, 108
welding stellite	5	oxy acetylene	5*			29
	7	MAG welding	175*			

* calculated arithmetic mean with assumption of normal distribution
** Co-air concentration was 50 μg Co/m^3 after improving the ventilation system

grinding may be higher than in dry grinding because of exposure to Co containing aerosols of cutting/cooling fluids (25, 63, 98, 107). High airborne Co concentrations were also found in Co refineries and during the production of Co containing diamond saws (31, 104).

The Co exposure in a Swedish hard metal plant was recently reported in an abstract (96). The air samples showed total dust and tungsten levels well below Swedish national standards but the Co concentration was sometimes high (extreme value 1.1 mg/m^3). Urine specimen collected at the end of the working week revealed U-Co levels of ≥15 μg/l in 29% of the workers (n=17) at the milling and mixing department.

Atomic absorption spectrometry (AAS) or X-ray fluorescence are advised for Co analysis in environmental samples (59). Blood Co (B-Co) and urine Co (U-Co) concentrations should be analysed with graphite furnace AAS with Zeeman background correction, which is a sensitive method (12, 102). More recently, inductively coupled plasma mass spectrophotometry (ICP-MS) was found to be a sensitive method for evaluation of environmental samples and U-Co. However, overestimation of U-Co was found at low concentrations (non-exposed persons). A high correlation between the formerly used AAS and the more recent ICP-MS methods suggest that both methods are reliable (115). Inductively coupled plasma

emission spectrometry and X-ray fluorescence appear to be too insensitive for determination of Co in biological matrices (38).

Uptake, distribution, elimination

The respiratory tract (dusts, fumes, aerosols or gases) and the digestive tract are the main routes of absorption (38). Absorption rates of Co or Co compounds depend on their solubility in biological media (65). The lung retention in 2 human volunteers after inhalation of cobalt (II,III) oxide particles varied between 64% and 75% after 90 days for particles with a diameter of 0.8 μm and 1.7 μm, respectively (10).

Human gastrointestinal absorption of orally supplied Co chloride varies between 1 and 50% and is influenced by the amount of Co given (71, 99). Uptake of Co chloride was higher than uptake of Co (II,III) oxide in humans and was higher in female than in male (15). Absorption was higher in patients with iron deficiency (71, 100).

Skin exposure to Co and Co compounds may result in significant dermal absorption. A dermal absorption rate of 2.2 μg Co/cm^2/hour was reported after applying $CoCl_2$ to human skin *in vitro* (111). For hard metal powder a dermal absorption rate of 0.033 μg Co/cm^2/hour for exposure of humans *in vivo* can be calculated from Scansetti *et al*. (91). Using these data and applying the ECETOC criteria for skin notation suggests that dermal exposure of workers to hard metal or $CoCl_2$ may result in significant systemic uptake, see further (83).

After intravenous administration of $^{60}CoCl_2$ to humans, ^{60}Co was mainly excreted in urine and to a lesser extent in faeces. The urinary excretion is characterized by a rapid phase of a few days duration (half-life 0.3 - 0.7 days) followed by 2 intermediate components (half-lives of 3-8 and 40-80 days) and a long-term component (half-live of about 800 days) (99). The mean urinary excretion of orally administered radioactively labeled $CoCl_2$ to humans (20 μmoles) was estimated to be 18% (range 9-23%) of the dose within 24 hours (100).

Oral administration of cobalt sulphate heptahydrate to pregnant rats has shown that Co can cross the placenta. Both maternal and fetal blood concentrations were higher after oral cobalt sulphate heptahydrate treatment compared to cobalt chloride hexahydrate (106). High Co concentrations in the fetal skeleton (and cartilaginous structures of the mother) were found after parenteral $CoCl_2$ administration to pregnant mice (38).

Biological monitoring

Urine, serum and whole blood Co concentrations of persons not occupationally exposed to Co are between 0.1-2 μg/l (38). There is a good correlation between exposure to soluble Co compounds (metal, salts and hard metal) and U-Co or B-Co levels when Co exposure is assessed by personal air sampling. These data can be used for assessing exposure on a group basis (67). U-Co is preferred above B-

Co since increases in airborne Co can be detected at lower levels (28, 38). According to Scansetti, *et al*. Monday end of shift U-Co gives an estimate of the exposure to hard metal on that day, while Friday end of shift samples are related to the cumulative exposure of the week (92). Poor correlations between Co in air concentrations and U-Co or B-Co were reported in Co oxide processing (67).

Toxic effects

Respiratory system

Mixed exposures of metallic cobalt, cobalt salts and cobalt oxides may cause asthma and obstructive lung function impairment. Hard metal and combined exposure of Co and diamond particles may give rise to interstitial lung disease and also to asthma.

An overview of the mechanism of toxicity of cobalt is discussed in the criteria document (83), see also the mutagenicity section.

Human data

Metallic Co, Co oxides and Co salts

A case-referent study (90) with 21 cases (workers with asthma) and 55 referents (workers without asthma randomly selected from the whole company) was carried out in a company, with complex exposure, that consisted of a cobalt, a zinc, and a sulfur plant. The asthma risk was increased for subjects exposed to Co (age adjusted OR= 4.8, 95%CI=2.0-11.7), i.e. for those working in the cobalt plant with exposure to cobalt sulphate or cobalt metal dust. Smoking was not associated with asthma. The levels ranged from less than 10 to 100 μg Co/m^3 in the cobalt plant (stationary sampling) and from 10 to 50 μg Co/m^3 in the cobalt roasting area (personal sampling). Five of 15 asthmatics regularly exposed to Co had a positive reaction to $CoCl_2$ in a provocation test and one had a positive reaction to dust from the Co roasting building. Pre-employment examination forms did not indicate that any of the cobalt workers had asthma before their current employment. The median average exposure time before onset of asthmatic symptoms was 11 month (range 2-36 month) for the 6 workers with positive provocation test. In 12 of the asthmatic cobalt workers, the asthma disappeared after removal from exposure. Two were later accidentally re-exposed to Co (water-soluble Co dust and metallic Co, respectively) and experienced typical clinical symptoms of asthma and had a positive provocation test to $CoCl_2$ (90). In a later study in the same plant, an additional case of occupational asthma with positive reaction to Co in a provocation test has been reported (62).

A cross sectional study among 82 workers of a Co refinery and 82 controls that were not exposed to lung irritants and were matched for age and sex, was performed. The workers were exposed to Co metal, oxides and salts at concentrations between 2-7700 μg Co/m^3 (geometric mean 125 μg Co/m^3, 164 exposure measurements) and had a mean exposure duration of 8 years. The exposed workers complained significantly more often of dyspnoea and wheezing,

especially the smokers. In addition, there was a significant positive relationship between current concentrations of Co in air or U-Co and dyspnoea during exercise. A significant relation was also found in the exposed group between the intensity of current exposure to Co (Co in air and U-Co) and the reduction of FEV_1/FVC (104).

No effect on the lung function was found in a cross sectional study among 224 workers who were exposed to Co metal, oxides and salts at concentrations less than 50 μg Co/m^3 and a mean exposure duration of 7.3 years. The referents (n=161) worked in a laboratory, office or power plant (90). Lung functions were also not impaired in a cross sectional study among 49 workers who were exposed to 520 μg Co/m^3 originating from Co metal and oxide. The mean exposure duration was 10.7 years. The referents (n=46) were not exposed to Co and were matched for smoking (72).

No interstitial lung disease (see below) was reported in these studies of workers exposed to Co metal, oxides or salts (62, 72, 104, 110).

Based on these studies it can be concluded that Co metal, oxides and salts may induce asthma (62, 90). A positive dose-effect relationship between Co exposure, originating from Co metal, oxides and salts, and obstructive lung function impairment was reported in one study (103). Two other studies did not find a relationship (72, 90). Interstitial lung disease was not found in workers exposed to Co metal, oxides and salts (62, 72, 104, 110).

Hard metal

Interstitial lung diseases are a group of diseases that are characterised by inflammatory changes in the lung interstitium. These diseases are often characterised by fibrosis and examples are allergic alveolitis, sarcoidosis, asbestosis, silicosis and hard metal disease. The signs and symptoms associated with these diseases include cough, phlegm, restrictive alterations, and decreased diffusion capacity. In severe cases of hard metal disease the lung function is severely impaired and death has been reported.

Sprince *et al*. performed a cross sectional study among 1039 hard metal production workers (101). Work-related wheeze occurred in 113 participants. The prevalence of work-related wheeze by present exposure category were ≤50 μg/m^3, 9.2%; >50 μg/m^3 to ≤100 μg/m^3, 18.1%; >100 μg/m^3, 15.4%. The odds ratio for work-related wheeze was 2.1 times (X^2=9.5, p<0.002) for present cobalt exposure exceeding 50 μg Co/m^3 compared with exposures ≤50 μg Co/m^3 after adjusting for current smoking, age, gender and race (no relative risk estimate could be calculated from the data given in the study). Abnormal chest radiographs was defined as showing profusion of small opacities ≥1/0 (ILO-classification) and occurred in 26 workers. The odds ratio for profusion ≥1/0 was 5.1 times (X^2=4.8, p<0.029) for average lifetime cobalt exposures exceeding 100 μg Co/m^3 compared with exposures ≤100 μg Co/m^3 in those with latency exceeding 10 years after adjusting for pack-years and age. Average lifetime exposure was defined as cumulative Co exposure divided by total duration of exposure. Interstitial lung disease was defined as profusion ≥1/1, FVC or DL_{CO} ≤70% and FEV_1/FVC%

≥75% and occurred in 7 workers (no control group). In two of the subjects with ILD, lung biopsies were made that showed interstitial fibrosis. Grinders of hard metal had a lower diffusion capacity for carbon monoxide compared to non-grinders, even though they were exposed to lower airborne Co concentrations (101). This phenomenon was also reported by Sjögren *et al*. and Kennedy *et al*. who found a higher prevalence of lung disease and restrictive lung function impairment among wet grinders (47, 98). Since wet grinders use coolants that often contain high Co concentrations, additional exposure via skin and/or gastrointestinal tract may be responsible for the increased toxic effects in grinders.

A cross sectional study was conducted on 425 workers and 88 controls who were working at one of three hard metal plants. The diffusion capacity for carbon monoxide was lower in exposed workers than in controls. This difference was more pronounced in women than in men. Slight abnormalities of chest radiographs were more frequent in exposed men than in controls (prevalence: 19.5% at a mean Co exposure of 30-220 μg Co/m^3 and 24% at 45-272 μg Co/m^3). Subjects with abnormal chest radiographs had a slight restrictive lung function impairment compared to matched controls. The differences could not be explained by smoking habits (70).

Symptoms and signs compatible with alveolitis were found in a case study among hard metal workers (98). In another case study, diffuse interstitial lung disease was reported among hard metal workers (17). In both studies, exposure measurements were not well described. No interstitial lung disease was found among 319 hard metal workers who were exposed to mean Co exposures up to 688 μg Co/m^3 during 1-29 years (55) and among 250 hard metal workers divided into six groups with average exposure concentrations varying between 2 to 60 μg Co/m^3 during 7-11 years, except for the dry grinders who's mean exposure duration was 4 years (2).

In a cross sectional Japanese study, 18 of 319 (5.6%) hard metal workers had asthma (the prevalence of asthma in the general population was not given). All nine patients with asthma who had a bronchial provocation test with Co chloride were positive. Mean Co exposures in four cases were 18, 24, >31 and >1203 μg Co/m^3 (52, 55). Shirakawa reported mean Co exposures between 7 – 227 μg/m^3 in 8 patients who developed occupational asthma. Four of these eight patients were atopic and seven showed bronchial hyperresponsiveness to methacholine. All patients had positive reactions to 1% $CoCl_2$ in the provocation test while the control subjects, including 6 asthmatic patients with high responsiveness to methacholine, showed no reaction. Tungsten was incapable of provoking asthma in challenge tests. Four patients had specific IgE antibodies to cobalt conjugated human serum albumin based on comparison of serum samples from 60 asthmatic patients and 25 asymptomatic workers in the same plant (97).

Impaired lung function caused by hard metal exposure was reported in many studies (2, 47, 53, 55, 70). Two key studies with low exposure levels will be discussed in detail.

In eight lumber mills that voluntarily participated in a cross sectional study, (118 saw filers, 90% participation) were compared with an external population of bus mechanics (number of bus mechanics not given). The saw filers were divided in 7 groups that performed different tasks, including wet grinding and dry grinding. Wet grinding was defined as grinding of tungsten carbide at least 10% of the time, for which at least 50% of the grinding was performed with a coolant; dry grinding was similarly defined, but required at least 50% of the grinding without a coolant. The full shift air Co concentration was determined in every filer between 1 and 4 times. Co was detected (detection limit 0.64 μg/m^3) in 62 of 278 samples (mean 9, max. 106, SD 20 μg/m^3). The within subject variability was very high; therefore exposure was estimated at group levels. Mean Co concentrations in used coolants from tungsten carbide grinding machines was 0.7 g/l (n=29). About three times the rate of cough, phlegm and wheeze related to work was reported by the filers compared to the bus mechanics. The wet grinders had significantly lower FEV_1 and FVC values compared to the other saw filers and the bus mechanics, whereas no differences were seen between other saw filers and bus mechanics. The effects on the wet grinders could not be explained by smoking habits. The estimated mean Co exposure for dry grinding was 5.4 μg Co/m^3 and for wet grinding 5.6 μg Co/m^3. Both Co exposure during wet grinding of tungsten carbide and duration of work were significantly associated with reductions in FEV_1 and FVC in the wet grinders. The airborne Co exposures were comparable for wet grinders and dry grinders and the authors speculate that dermal absorption of Co in the wet grinders might have contributed to systemic uptake. Other speculations to explain the different effects seen in dry and wet grinders were that the coolant might have an adjuvant effect or might change the state of Co. Wet grinders of other metals, eg. stellite and mild steel, using the same coolant, did not show reductions in lung function (47, 107).

In a cross sectional study, hard metal workers from four major Swedish hard metal industries were divided in six different exposure groups according to job category (1, 2). The mean cobalt exposure duration was 7–11 years, except for dry grinders who had a mean duration of 4 years. Office workers in the same industries were used as controls; these were matched pairwise to each exposure group by sex, age, length, and smoking habits. Exposure levels were based on personal monitoring data (breathing zone) from the same work places. Several symptoms were more common in the cobalt exposed workers (Table 3). According to an interview survey, prevalence of irritation of eyes, nose or throat was significantly elevated in all relevant exposure groups (given mean exposure levels: 3–60 μg/m^3), but with no clear dose-response (Table 3). Cough with phlegm was also significantly increased in the lowest exposure group, but with an inconsistent dose-response pattern. Chronic bronchitis was significantly more frequent in the highest (60 μg Co/m^3), but not in lower exposure groups (Table 3). These chronic symptoms were more common among smokers. Details about the interview survey are not reported. Lung function tests of the workers in the

Table 3. Symptom frequency (% exposed/% control) in different groups occupationally exposed to cobalt in four hard metal plants in Sweden. Adapted from Alexandersson (2)[1].

Job type (exposure group)	Office work (control)	Quality inspection[2]	Surface grinding	Powder handling	Wet grinding	Dry grinding	Powder handling
Mean exposure[3] (μg Co/m^3)	0.8 – 0.9	2	3	5-10	8	12	60
Irritation of eyes, nose or throat	-	**18/0**	**35/7**	**27/0**	**35/4**	**32/0**	**40/2**
Breathlessness or feeling of heavy to bread during work	-	9/0	3/0	10/0	**16/0**	16/0	**24/0**
Cough without phlegm	-	14/4	14/17	20/3	**23/7**	8/8	8/10
Cough with phlegm	**-**	**21/0**	**28/3**	10/0	**23/5**	4/4	**35/6**
Chronic bronchitis[4]	-	4/0	0/0	0/0	5/0	0/0	**11/0**
Chest tightness	-	34/18	24/21	33/17	**46/18**	32/16	27/18
Number of subjects[5]	-	44	29	30	57	27	63

[1]Bold figures indicate significant difference between exposed group and control group ($p \leq 0.05$).
[2]According to authors, symptoms in this group is probably due to selection and not related to cobalt exposure.
[3]Previous exposures were reported to have been higher.
[4]Diagnosed by physician.
[5]Exposed and controls were pair wise matched, considering sex, age, height, and smoking habit. Asthmatics were excluded.

highest exposure group (60 μg Co/m^3) revealed significant impairment in FEV_1, FEV%, and MMF (maximum midexpiratory flow) compared to paired controls and in FVC, FEV_1, and MMF over the working week. In dry grinders exposed to 12 μg Co/m^3, tendencies to impairment in FVC compared to controls was seen and in wet grinders, exposed to 8 μg Co/m^3, in FEV_1 and MMF over the working week. No significant impairment of lung function parameters was found in the other exposure groups. It should be noted that exposure measurements were the most recent ones, performed within a couple of years (no further details given). Exposures were markedly higher in the past (2). Thus, the chronic symptoms may have been caused by earlier, higher exposures. A 5-year follow-up of 27 workers showed additional FEV_1 impairment in smokers. The mean exposure of these workers decreased from 80 to 30 μg Co/m^3 during this period (6). A dose-effect relationship was demonstrated between U-Co and FEV_1 and between B-Co and FEV_1 only in smokers (5).

Male smoking hard metal exposed workers had decreased values of FEV_1, peak expiratory flow and forced expiratory flow at 50% of vital capacity. This effect was only seen in hard metal workers who smoked. The workers were stratified in three exposure groups (≤50, 50-100 and ≥100 μg Co/m^3). Smoking habits were stratified in current smoker, ex-smoker and non-smoker (54).

Sixteen ex-factory workers with diagnosed hard metal disease and with previous cobalt and solvent exposure were tested for memory deficits. They had been exposed for 2–35 years (levels not reported) and removed from work 1 month to 8 years prior to testing. Results demonstrated deficits in the allocation of attentional resources and in short-term verbal memory (42). If these reported deficits are secondary to hypoxia due to lung function impairment or a direct effect of Co on the CNS remains to be clarified.

In summary, irritative effects (eyes, nose and throat) from hard metal exposure has been reported at a mean exposure level of 3 μg/m^3 (2). ILD from hard metal exposure has been reported (17, 70, 98, 101). No epidemiological data are available on ILD caused by tungsten(carbide) without Co. Restrictive lung impairment was found among wet grinders exposed to mean Co concentrations of 5.6 μg/m^3 (47). Several studies reported increased lung toxicity for wet grinders compared to dry grinders, which may be a result of additional dermal Co exposure from Co containing coolants (47, 98, 101). Hard metal can also induce asthma (52, 55, 97).

Co containing diamond polishing dust

Demedts *et al*. reported 5 cases of interstitial lung disease among diamond polishers using Co containing abrasive disks. No exposure measurements were available (22).

Bronchial asthma among diamond polishers was described in 3 cases. The patients had worked with Co containing abrasive disks. All three patients were positive in a cobalt inhalation challenge test (32).

In a cross-sectional study among 194 diamond polishers working with Co-containing disks and 59 controls who worked with disks without Co, three dose groups were formed. The Co exposure of the controls varied between 0.08 and 1.5 μg/m^3. The mean Co exposure in the low and high exposure group was 5.3 μg Co/m^3 and 15 μg Co/m^3, respectively. Mean U-Co concentrations for the three dose groups were 2, 7 and 21 μg Co/g creatinine respectively. FVC and FEV_1, but not FEV_1/FVC, were significantly lower in the high exposure group compared to the low exposure group. This was also found when the high exposure group was compared with the pooled low Co and control group. The effects were more pronounced in women. The differences were not due to differences in smoking habits. Both exposure and health measurements were cross sectional, thus a healthy worker effect may have underestimated the effect of Co exposure on lung function (78).

A decrease of FVC and FEV_1 (but not FEV_1/FVC) was reported in a cross-sectional study among 48 workers producing diamond-Co circular saws and 23

controls. This was true for smokers and non-smokers. Non-smokers who were exposed for more than 5 years had a tendency to an obstructive effect. Exposures varied between 6.2-2875 μg Co/m^3 (31).

It can be concluded that combined exposure to Co and diamond particles leads to interstitial lung disease and induces asthma. Restrictive lung impairment was reported among workers exposed to both diamond particles and a mean Co concentration of 15 μg Co/m^3.

Vitallium

Exposure to vitallium dust, an alloy of Co (56-68%), chromium and molybdenium, has been associated with the development of pneumoconiosis in dental technicians (77, 94, 95).

In a cross-sectional study 37 dental technicians with at least 5 years (range 5-36 years) exposure to vitallium showed a restrictive lung function impairment compared with historical reference material. A dose-response relation between exposure to vitallium dust in hours per week and reductions in both FVC and FEV_1 was found. The reduction was more pronounced in smokers than in non-smokers and ex-smokers. Six (16%) of the 37 dental technicians showed radiological evidence of pneumoconiosis. Dust measurements were carried out for those technicians (10 subjects) who had a minimum weekly working time with vitallium of 20 hours. Co concentrations in the air between 25 and 1600 μg Co/m^3 were measured when no local exhaust was available. When local exhaust ventilation was available the Co concentrations were lower than 25 μg Co/m^3 (95). Dental technicians are exposed to a complex mixture of dust particles and it is not possible to make a distinction between asbestos or silicon carbide fibres or other elements such as aluminium silicate, quartz, corundum, or vitallium as a single causative agent (95).

The mixed dust pneumoconiosis associated with vitallium exposure should not be confused with interstitial lung disease caused by hard metal dust (66), since vitallium is a homogenous alloy and hard metal is not. Co in vitallium is remarkably stable in biological fluid, whereas Co in hard metal is rapidly solubilized and cannot be found in lung or bronchoalveolar lavage fluid of patients. No giant cells or desquamative alveolitis have been seen in the dental technicians (66).

Co-Zn silicate

Impaired lung function was reported in a cross sectional study after exposure to Co-Zn silicate. The forced expiratory flow rate at 25% and 50% of the vital capacity was decreased. Co exposures were not well defined since technical adjustments to the fume cupboards were made during the study (88). In this study, the number of smokers was higher in the exposed workers than in the controls and "smoking may be a confounder" (15).

Animal data

Inhalation exposure of rats to Co sulfate heptahydrate during 13 weeks affected the lungs. Inflammation (histiocytic infiltrates) of the lung was found at concentrations equal or higher than 400 μg Co/m^3 and more severe inflammation was found at a concentration equal or higher than 1100 μg Co/m^3. Bronchiolar regeneration, peribronchiolar and septal fibrosis were seen at concentrations equal or higher than 11000 μg Co/m^3 (13, 80). Rats who were exposed to Co sulfate heptahydrate aerosols during 2 years developed alveolar inflammation and interstitial fibrosis at 100 μg Co/m^3. Mice were less sensitive in this study (14, 81).

Rabbits were exposed to 400 or 2000 μg Co/m^3 as Co chloride aerosols for 14-16 weeks. At the higher Co chloride concentration an increased number of macrophages in bronchoalveolar lavage fluid was found. Lysozyme activity and oxidative metabolic activity of macrophages were increased in both exposed groups (41).

Decreased lung compliance and microscopic evidence of interstitial fibrosis by an increase of septal collagen were found in miniature swine after inhalation exposure to 100 μg/m^3 Co powder during 3 months (48).

Rats exposed via a single intratracheal instillation of a mixture of Co and tungsten carbide particles in saline (10000 μg/kg bw, corresponding to 600 μg Co/kg bw), showed acute alveolitis during at least 1 month. No fibrosis was seen after 4 months. Exposure to either cobalt (600 μg Co/kg bw) or tungsten carbide (10000 μg/kg bw) resulted in very modest effects. Extension of the treatments to 4 administrations at 1 month interval resulted in interstitial fibrosis after exposure to the mixture of Co and tungsten carbide. No effects were seen after exposure to Co or tungsten carbide alone (56).

Hamsters exposed to Co(II)oxide aerosols (8000 μg Co/m^3) up to 22 months developed pneumoconiosis from early on. This was characterised by interstitial pneumonitis, diffuse granulomatous pneumonia and fibrosis of alveolar septa (114).

Skin

Skin exposure to cobalt and cobalt compounds may occur in the industries already mentioned in Table 2, and also in concrete construction work since cement contains Co. Co is one of the major contact allergens, and 4% of patch-tested dermatitis patients are patch-test positive to $CoCl_2$ (43). Knowledge about sources of sensitisation and elicitation is however limited. Solitary Co allergy, without simultaneous contact allergy to nickel or chromate, is seen mainly among hard-metal workers and in glass and pottery industry. 5% of 853 hard-metal workers in a plant were allergic to cobalt (30). Although Co sensitivity generally occurs simultaneously with allergy to other metals (nickel and/or chromium), this is not believed to be due to a cross reactivity phenomenon but rather to combined exposure (37, 61). $CoCl_2$ was classified as a grade 3 allergen in a human

maximation test (highest: 5), and as grade 5 allergen in a guinea pig maximisation test. The animals did not react to nickel sulfate or chromate which is in agreement with the theory of multiple sensitisation rather that cross reactivity (60, 61, 112, 113). Single cases of photocontact dermatitis due to cobalt have been described (89).

Thyroid gland

Co therapy of patients with anemia caused thyroid hyperplasia associated with thyroid hypofunction. The applied doses were 3000-4000 μg Co/kg/day during 3-7.5 month (50). Decreases in plasma T3 and T4 and an increase in TSH were found in 82 workers exposed to 2 – 7700 μg Co/m^3 during 8 years (104). In contrast, an increased T4, marginally reduced T3 and unaltered TSH were found in 25 plate painters exposed to semi soluble Co-Zn silicates. The mean U-Co of these workers was 1.17 μg/mmol creatinine. Co-air measurements of about 50 μg/m^3 were reported, but no description was given of the measurement strategy and the analysis of the samples (87). Overall, the data on toxic effects on the thyroid gland from occupational exposure to Co are inconclusive.

Cardiovascular system and blood and blood-forming organs

Cardiomyopathy was found in heavy beer drinkers after Co chloride or Co sulfate was added to beer. The estimated daily intake was 6000-8000 μg Co. Remarkably, patients who were treated with Co chloride up to 100 000 μg/day did not develop cardiomyopathy. Alcohol intake and bad nutritional status (low protein) may have contributed to the cardiomyopathy in the beer drinkers (65, 93). Cardiomyopathy was also described in 2 workers exposed to high concentrations of dust from Co-containing ores (39) and in some cases in which no exposure data are available (11, 46). Thirty hard metal workers exposed for 10-15 years had a normal cardiac function at rest. In hard metal workers with abnormal chest X-ray findings, the right ventricular ejection fraction was reduced with exercise. This was probably due to fibrotic pulmonary disease and early cor pulmonale (36, 93). Alexandersson and Atterhög compared 42 dry grinders, 43 wet grinders and 61 powder handlers with 126 controls. The mean Co exposures of the groups were 10 μg Co/m^3 for both the dry and the wet grinders, 60 μg Co/m^3 for the powder handlers and no exposure for the control group. Only the wet grinders had ST- and T-depressions in the ECG with an overfrequency of ectopic beats but they had no pulmonary dysfunction. In the other groups no effects on the heart function were found (3). The small ECG changes in the wet grinders had disappeared after 4 weeks vacation (4).

No excess mortality from diseases of the circulatory system were found in a French cohort study among Co production workers (born in France), SMR=0.80, 95%CI 0.36-1.51 (73), and in a cohort study among 7459 hard metal workers, SMR=0.88, 95%CI 0.75-1.03 (74). This is in contrast to a Swedish cohort study in which increased mortality from ischemic heart disease was found in hard metal

workers. The effect was only found in the highest exposure group (up to 11000 μg Co/m^3) with more than 10 years employment who had died more than 20 years after the beginning of exposure (16 cases vs 9.4 expected, SMR=1.69, 95%CI 0.96-2.75 (35). The calculated SMR's are based on national rates.

In the past, Co was used as a therapy to increase red blood cell number, hemoglobin, and hematocrit. The applied oral doses were 6200-12400 μg Co/day during 12-30 weeks (26). In a cross sectional study among 82 Co refinery workers and 82 age matched controls, a reduction in hematocrit and hemoglobin was reported, which could not be explained by the authors (104). Co increases erythropoietin concentrations by simulating hypoxia (7, 33, 65).

Several studies shows that rats, pigs and guinea pigs develop cardiomyopathy after oral administration of Co salts. The applied doses varied between 3000 and 100 000 μg Co/kg/day during 3 days up till 20 weeks. The results also suggest that thiamine or protein deficiency may exacerbate this condition (27). ECG changes were reported in miniature swine after inhalation exposure to 100 μg/m^3 Co powder during 3 months. The ECG changes were loss of QRS voltage indicating a decrease in ventricular contraction and T-wave changes indicating repolarisation abnormalities (48).

Male B6C3F1 mice were exposed to cobalt sulfate heptahydrate (mass median aerodynamic diameter 1.5-1.8 μm) by inhalation of 3.0 mg/m^3 (corresponding to 630 μg Co/m^3) for 2 years. Arteritis was detected in heart and kidney (75).

Optic atrophy and deafness

Bilateral deafness and visual failure were reported in a case study of a 48 year old employee exposed to Co powder for 20 months, working 50 hours a week. No exposure measurements are available. The complaints disappeared after he stopped working. Optic atrophy was found in a patient who received a total dose of 73 g $CoCl_2$ in 3 years. Bilateral deafness due to nerve damage was found in a patient who received a daily dose of 100 000 μg $CoCl_2$ for 6 months. Four out of 16 patients who were treated with $CoCl_2$ complained of tinnitus after 4-16 weeks of therapy. The effect disappeared after stopping the therapy (69).

Mutagenicity, carcinogenicity

The carcinogenic potential of Co and its compounds was evaluated by IARC in 1991 (38). The overal evaluation was that Co and its compounds are possibly carcinogenic to humans (group 2B). This evaluation was based on *inadequate evidence* in humans and *sufficient, limited or inadequate evidence* (depending on the type of cobalt compound) in experimental animals.

Production of activated oxygen species is a common mechanism of genotoxicity for cobalt(II) ions, cobalt metal and hard metal (Co plus tungsten carbide), and cobalt and cobalt compounds are believed to act as indirect genotoxicants due to the formation of activated oxygen species. In addition,

Co(II) ions are known to inhibit DNA repair. Co(II) ions are formed during the production of activated oxygen species from cobalt and hard metal (68).

In a population (n= 78) of workers selected for Cd exposure, but also exposed to Co (mean air level: 2.0 μg Co/m^3, state of Co not defined) and Pb, levels of DNA-SSB (single strand breaks) in mononuclear blood cells correlated strongly to Co levels in air (personal sampler) and in blood. Increased levels of SSB was recorded at Co levels of between 4–10 μg/m^3. An inhibition of repair activity of DNA adducts (8-oxoguanine) in blood from these workers was also reported, but referred to as unpublished data. The authors conclude that Co was the strongest determinant, but that interactions with Cd and/or Pb seem likely (34).

Co ions

Intratracheal instillation of Co chloride to hamsters caused thiol oxidation in lung tissue indicating oxidative stress (79). Intraperitoneal administration of Co(II) ions to rats produced oxidative DNA damage caused by hydroxyl radicals in renal, hepatic and pulmonary chromatin (44). Chromosomal aberrations were also found in mice after oral administration of Co chloride (82). DNA breakage was shown in human lymphocytes exposed *in vitro* to non cytotoxic Co chloride concentrations (21).

Inhalation exposure of rats and mice to Co sulphate heptahydrate during 13 weeks caused squamous metaplasia of the larynx (13, 80). Co sulphate heptahydrate was also studied in a 2-year inhalation study. Incidences of alveolar/bronchiolar neoplasms were significantly increased compared to controls in both male and female rats and mice. Benign, complex or malignant adrenal pheochromocytoma were significantly increased in male and female rats. The overall conclusion was that there is some evidence of carcinogenic activity in male rats, clear evidence in female rats and clear evidence in male and female mice (14, 81). The European Union has classified Co chloride and sulphate as substances which may cause cancer in humans by inhalation (C2 carcinogens) (68).

It can be concluded that there is evidence that Co(II) exerts carcinogenic effects in animals. No evidence about genotoxicity or carcinogenicity is available in humans.

Co metal and Co oxides

No increase in genotoxicity biomarkers in lymphocytes was found in 35 workers from Co refineries compared with matched controls. The average Co exposure of the workers was 20 µg Co/m^3 (20).

Two cohort studies among workers in an electrochemical plant producing Co and sodium were performed. The first study (1950-1980) found a significant excess of lung cancer among workers in the production of Co (SMR 4.66; 95%CI 1.46-10.64) but the number of cases was only 4. Smoking was not taken into account (76). Extension of the follow up (1981-1988) by the same authors did not confirm the hypothesis of a relation between lung cancer and Co exposure (SMR

0.85; 95%CI 0.18-2.50). No worker died of lung cancer during the extension of the follow up. Reevaluation of the 4 cases in the first study in which general practitioners' records were used to set the number of lung cancers showed that there was no death certificate for one of the 4 cases. This means that there were only 3 cases of lung cancer in the latter study (73).

It may be concluded that the epidemiological studies are insufficient to evaluate the carcinogenic potential for Co metal alone. According to IARC, there is sufficient evidence that Co(II) oxide is carcinogenic in animals and inadequate evidence that Co(II,III) oxide is carcinogenic in animals (38). No study examining the genotoxic or carcinogenic activity of Co oxides was found in the literature published since the 1991 IARC evaluation.

Hard metal

No increased genotoxic effect in lymphocytes was found in 29 hard metal workers who were exposed to a mean Co concentration of 20 $\mu g/m^3$ (20). Hyperplasia of type II alveolar epithelial lining cells was found in patients with severe restrictive ventilatory defects (9, 19). A retrospective cohort (1951-1982) of 3163 hard metal workers showed an excess mortality from lung cancer only in workers with more than 10 years employment who had died more than 20 years after the beginning of exposure (7 cases observed versus 2.5 expected; SMR 2.78, 95%CI 1.11-5.72). A dose-response relationship was not found (35). An increased mortality from lung cancer was found in a French follow up from 1956-1989 among 709 hard metal workers (10 cases observed, SMR 2.13, 95%CI 1.02-3.93). This excess was highest among workers employed in the areas with Co exposures higher than 50 $\mu g/m^3$ (6 cases observed, SMR 5.03, 95%CI 1.85-10.95). Lung cancer mortality could not be explained by smoking alone (57). Extension of this study to a cohort of 7459 workers from all hard metal plants in France (covering years 1968 to 1991) showed again that mortality from lung cancer in this cohort was borderline significantly increased (63 cases observed, SMR 1.30, 95%CI 1.00-1.66). Mortality from lung cancer increased slightly with time since first employment. A nested case control study (61 cases and 180 controls) showed a twofold lung cancer risk among workers exposed to Co and tungsten carbide when the exposures during the last 10 years were ignored (OR=1.93, 95%CI 1.03-3.62). The odds ratio increased with cumulative exposure and duration of exposure. Smoking could not explain the excess of lung cancer (74). A historic cohort study (117) was set up in one of the sites already included in the study of Moulin *et al*. (74). Full job histories were available in contrast to the study of Moulin that relied on job exposure matrices. The results of the study (117) were in agreement with the results of Moulin *et al*. (74).

The above human mortality studies support a carcinogenic effect of hard metal particles.

Other Co compounds

A retrospective cohort study among 874 women exposed to 65-8600 μg Co/m^3 of an insoluble Co-aluminate dye showed no increased incidence of lung cancer compared to 520 non-exposed controls (15, 108).

Reproduction

No information about reproductive effects of Co is available in humans.

In a 13-week inhalation study, mice of each sex were exposed to cobalt sulphate aerosols, 3, 10 or 30 mg/m^3 (1.1, 4, 11 mg Co/m^3), 6 hours per day, 5 days per week. Mean body weights of mice exposed to 30 mg/m^3 were lower than those of controls throughout the study and two of 10 males in this group died before the end of the study. Sperm motility was decreased in male mice at all three concentrations tested, in a dose-dependent manner, compared to unexposed controls and increased numbers of abnormal sperm and decreased testis and epididymal weights occurred in mice exposed to 30 mg/m^3. In female mice, the length of the oestrous cycle was increased in the highest dose group. (13). Rats exposed in the same way showed no effects on the reproductive system (13).

Orally administered cobalt chloride to male mice showed decreased testicular weight, decreased sperm concentration, impaired sperm mobility and abnormal spermatic nuclei (8, 18, 85). Preimplantation losses were found when female mice were mated with cobalt chloride treated male mice (86). The cobalt concentrations in these studies were 20 mg Co/kg bw per day or more and the rats were treated for 10-14 weeks.

The effects on fetal development of cobalt sulphate administrated by gavage to pregnant mice, rats and rabbits was studied by Szakmáry *et al*. (106). In mice and rats the treatment significantly increased the frequency of fetuses with retarded body weight and produced skeletal retardation (in rats in a dose dependent manner). A few anomalies in the urogenital system were observed in the treated groups. Also skeletal malformations, cranium (mice) and vertebra (mice and rats) were reported. In rabbits no malformations were seen (106). No firm conclusions about the teratogenic effects of cobalt sulphate in experimental animals can be drawn from this study since it contains several inconsistencies regarding, e.g. data presentation, maternal toxicity and dose-response relationships.

Paternain *et al*. reported no embryotoxic or teratogenic effects in rats after oral administration of $CoCl_2$ to pregnant rats at concentrations up to 100 mg/kg/day on day 6-15 of gestation (84). A single injection in the tail vein of pregnant mice of $CoCl_2$ (dose: 1.2 mg Co/kg bw on day 8 of pregnancy) showed an interference of the metal with the fetal skeletal ossification (116).

Lowered birth weight after oral administration of cobalt sulphate to pregnant rats (25 mg Co/kg bw per day, day 1-21 of gestation) compared to controls has been reported (106). Also a reduction of the number of litters 5 days after birth and lowered ability in a swimming test (day 18 to 22 after birth) was found. Domingo *et al*. also found a lowered birth weight and reduction of the number

of litters and a dose-dependent delay in the growth of living pups after oral administration of cobalt chloride to pregnant rats (12, 24, 48 mg/kg bw per day, from day 14 of gestation through day 21 of lactation) (23).

Dose-response/dose-effect relationships

In Table 4 and 5, human data on inhalation exposure are presented. Table 4 summarizes the effects of exposure to Co metal, salts and oxides; Table 5 gives an overview of combined exposure of Co and other compounds.

Irritative effects have been shown after exposure to Co containing dust. Workers in the hard metal industry complained of irritation of eyes, nose and throat at a mean exposure of 3 μg Co/m^3 and diamond polishers at a mean exposure of 15 μg Co/m^3 (Table 5).

Induction of asthma has been reported after mixed exposure to water soluble Co and Co metal as well as to hard metal at an exposure level of 10-50 μg Co/m^3 (Table 4 and Table 5), but no conclusions about dose-response relationships can be made.

ILD has been reported from hard metal exposure and restrictive lung impairment was found among wet grinders exposed to mean Co concentrations of 5.6 μg/m^3 (Table 5). In this case the state of absorbed Co might have been altered by the coolant, or skin absorption might have been involved.

Another study reports reduction of FEV_1 and MMF among (hard metal) grinders at 8 μg/m^3, but no reduction among grinders at 3 μg/m^3 (Table 5). Both groups were exposed to cutting fluids.

A reduction of FEV_1 and FVC has been reported among diamond polishers exposed to 15 μg/m^3 when compared with polishers exposed to 5.3 μg/m^3 (Table 5).

An increased risk of abnormal chest radiographs (profusion ≥1/0) has been reported in hard metal workers at an average life time exposure of >100 μg Co/m^3 compared to hard metal workers with an average life time exposure of ≤100 μg Co/m^3 (Table 5).

No epidemiological data are available on ILD caused by tungsten(carbide) without Co. Animal studies, however, support an interaction between tungsten carbide and Co in the development of ILD.

Increased levels of DNA damages (SSB) has been reported in workers exposed to 4-10 μg Co/m^3, but interactions with Cd and Pb seem likely.

Animal inhalation studies indicate alveolar inflammation, interstitial fibrosis and ECG changes at a level of 100 μg Co/m^3 and testicular toxicity (decreased sperm motility) at 1100 μg Co/m^3 (Table 6).

Conclusions

The critical effect of occupational exposure to Co and Co compounds is irritation of eyes, nose and throat. This was found at a mean Co exposure of 3 μg Co/m^3. Other effects on the respiratory system appear at slightly higher levels. Impair-

ment of lung function was seen among hard metal grinders at 5.6 but not at 3 μg Co/m^3. Co and Co compounds can induce occupational asthma, but no conclusions about dose-response relationships can be made. Pneumoconiosis has been associated with exposure to hard metal dust, vitallium dust and combined diamond and cobalt dust. Several studies report a positive interaction between the effects of Co exposure and smoking (chronic bronchitis and impaired FEV_1).

Co is genotoxic presumably via an indirect mechanism involving reactive oxygen species. Genotoxic potential *in vitro* has been shown for Co ions and Co metal particles.

There is evidence that Co ions and Co oxides are carcinogenic in animals. One study indicates that inhalation of hard metal dust is carcinogenic in humans.

Co and Co compounds are skin sensitisers. Dermal exposure to hard metal and cobalt chloride may result in significant systemic uptake of cobalt.

Table 4. Effects in humans exposed to Co metal, oxide or salt.

Concentration (μg Co/m^3)	Exposure/duration	Effects	Ref.
case 1: <6000-84000 case 2: 64000-103000	Dust from cobalt-containing ores case 1: 26 months case 2: 2 months	Cardiomyopathy in 2 workers	39
10-50	Co roasting, leaching, packing water soluble Co, Co metal; minimum duration: 6 months, 2-4 h/day plus maintenance operations for all factories	Case-referent study Age adjusted odds ratio for asthma 4.8 (95%CI: 2.0-11.7) 21 cases with asthma, 55 randomly selected workers without asthma 6 of 15 Co workers with asthma were positive in a challenge test with $CoCl_2$ or dust of Co roasting	90
2–7700 geometric mean: 125 70% >50 25% >500	Co metal, oxides, salts; Mean exposure duration was 8 y (0.3-39.4 y)	Significant relationship between the level of current exposure to Co (Co-air and U-Co) and reduction in FEV_1/FVC ratio No signs of pulmonary fibrosis Decreased T3 and T4, increased TSH in plasma (82 workers, 82 age matched controls)	104
4–10	Pigment prod., battery work, recycling electronics Cd and Pb exposure	DNA damage (SSB)	34

Table 5. Effects in humans exposed to hard metal dust, vitallium or diamond polishing dust.

Concentration (μg Co/m^3)	Exposure/ duration	Effects	Ref.
up to 1600	Vitallium, 5 y or more	Reduction FVC and FEV_1 (37 workers, no control group)	95
45-272 (powder) 30-220 (presses)	Hard metal, 13-14 y	Slight abnormalities in chest radiographs Lower FVC, FEV_1, carbon monoxide diffusion capacity (425 workers, 88 controls from mechanical workshops, warehouses and shipping departments)	70
mean Co conc. in 4 cases of asthma 18, 24, >31, >1203	Hard metal, latency period: 3 months to 10 y	Occupational asthma in 18 workers (319 workers, no control group)	55
≥100 "average lifetime exposures"	Hard metal, latency >10 y	The relative odds of abnormal chest radiographs (profusion ≥1/0) was 5.1, compared with average lifetime exposures ≤100 μg Co/m^3	101
AM: 60	Hard metal, 7-11 y	Reduction of FEV_1, FEV% and MMF compared to paired controls (63 workers, 63 controls). Reduction of FVC, FEV_1, and MMF over the working week (73 workers)	2
0.7-43 AM: 15	Diamond polishing dust of Co-containing abrasive disks, duration not given	Irritation of eye, nose and throat Reduction of FEV_1, FVC (unchanged ratio) compared to a lower exposed group (92 workers)	78
2-34 AM: 10	Hard metal, wet grinders, 7-10 y	ST- and T-depressions in ECG and overfrequency of ectopic beats	3
AM 8	Hard metal 7-11 y	Decreased FEV_1 and MMF between Monday morning and Friday afternoon Cough with/without phlegm, chest tightness, breathlessness (67 workers)	2
5.6, estimated exposure during wetgrinding	Hard metal, mean duration: 6.9 y (0.5-22 y)	Cough, phlegm and wheeze Reduction of FEV_1 and FVC (118 workers, number of controls [bus mechanics] is not given)	47
AM 3	Hard metal 7-11 y	No effect on FEV_1 and MMF between Monday morning and Friday afternoon (32 workers)	2
AM 3	Hard metal 7-11 y	Irritation of eyes, nose and throat (44 workers and 44 controls [office workers])	2

AM: Arithmetic mean
"Average lifetime exposure": fraction of cumulative Co exposure and total exposure duration.

Table 6. Effects in animals from animal inhalation studies.

Concentration (μg Co/m^3)	Exposure/ duration	Species	Effects	Ref.
400 or 2000	Co chloride 6 hr/day, 5 days/week, 14-16 weeks	rabbits male	Increased number of macrophages in the high dose group Increased lysozyme activity and oxidative metabolism in macrophages in both dose groups	41
8000	Co(II)oxide 7 hr/day, 5 days/week, from age 2 months until natural death up to 22 months	hamster	Interstitial pneumonitis, diffuse granulomatous pneumonia, fibrosis of alveolar septa	114
11000	Co sulfate 6 hr/day, 5 days/week, 13 weeks	mice	Decrease of testis and epididymal weight Increase of abnormal sperm Increase of oestrous cycle	13
1100			Decrease of sperm motility	13
11000	Co sulfate 6 hr/day, 5 days/week, 13 weeks	rat	Bronchiolar regeneration Peribronchiolar and septal fibrosis	13
1100			Inflammation of the lungs	13
630	Co sulfate 2 years	mice (male)	Arteritis in heart and kidney	75
400	Co sulfate 6 hr/day, 5 days/week, 13 weeks	rat	Histiocytic infiltration	13
100	Co metal particles, 6hr/day, 5 days/week, 3 month	Miniature swine	ECG changes	48
100	Co sulfate 6 hr per day, 5 days/week, 104 weeks	rat	Alveolar inflammation, Interstitial fibrosis	14, 81

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Consensus Report for Synthetic Inorganic Fibers

December 3, 2003

This document is an update of a previous Consensus Report published in 1988 (60). The Criteria Group also published a Consensus Report for Synthetic Inorganic Fibers in 1982 (52). Some of the terms, concepts and synonyms used in works on fibers are defined in Appendix 1, and international classifications are presented in Appendix 2.

Classification of synthetic inorganic fibers

Synthetically produced fibers of mineral origin (synthetic mineral fibers, or SMF) are generally grouped under the term Man-Made Mineral Fibers (MMMF). They may be either crystalline or non-crystalline. The term Man-Made Vitreous Fibers (MMVF) is sometimes used for fibers having a non-crystalline structure (*vitreous* = glass-like). The MMVF include the large product categories continuous fibers, slag wool, glass wool, rock wool, refractory ceramic fibers and special-purpose fibers. In Sweden the term *syntetiska oorganiska fibrer* (synthetic inorganic fibers) is used in an occupational health context, and covers all such fibers as well as non-silicate ceramic fibers (such as aluminum oxide fibers) and some fibers with crystalline structure (such as synthetic graphite fibers).

The *vitreous synthetic inorganic fibers* comprise fibers produced from melted glass, slag, rock (diabase/basalt), or kaolin clay (or aluminum oxide and quartz), by various processes. Differentiation between "continuous fibers," "mineral wool fibers" (glass, rock and slag), "refractory ceramic fibers" and "special-purpose fibers" may be based on the raw materials, the production method, the characteristics of the fiber, and/or the area of use. *Continuous fibers* are glass fibers of a specific diameter drawn from molten glass through a nozzle. They are usually more than 6 μm in diameter and are not thinner than 3 μm. Glass wool, rock wool and slag wool are often grouped together under the term "mineral wool" or "insulation wool," and the non-crystalline fibers from these products are commonly referred to as *mineral wool fibers*. The term *refractory ceramic fibers* (RCF) refers to fibers produced from molten aluminum silicate. These fibers are widely used as a substitute for asbestos in various high-temperature applications. When the non-crystalline refractory ceramic fibers (aluminosilicate fibers) are exposed to high temperatures their atomic structure changes, and new crystalline forms can be created. Mullite (crystalline aluminum silicate) is formed at about 1000 °C, and as the temperature rises above about 1080 °C cristobalite (crystalline silicon dioxide) is also formed. This transformation reaches its maximum at about

1200 °C, and above this temperature the mullite proportion remains relatively constant, whereas the cristobalite gradually disintegrates as the temperature approaches 1400 °C (38, 39, 48). The transformation to cristobalite is slower than the transformation to mullite (84). The term *special-purpose glass fibers* applies primarily to extremely fine glass fibers (microfibers) for specialized applications (e.g. in filters). There are also coarser special-purpose fibers, but their use is extremely limited.

The National Swedish Chemicals Inspectorate has adopted the European Commission classification (25) and defines synthetic vitreous inorganic fibers as silicate fibers with random orientation and a chemical composition in which the content of alkali metal oxides and alkaline earth metals is more than 18% by weight for mineral wool fibers, and up to 18% by weight for refractory ceramic fibers and special-purpose fibers (50). The International Program for Chemical Safety (IPCS) (44) has gone somewhat further, and divided the vitreous synthetic inorganic fibers into nine different categories based on chemical composition (total content of alkali metal oxides and alkaline earth metals), fiber orientation (parallel or random) and more, or less, than 1% by weight of various fiber fractions relevant to health. These fiber fractions are < 4 μm in diameter for continuous fibers, and < 3 μm or < 1 μm in diameter for other synthetic inorganic fiber fractions. For both coarse and fine special-purpose fibers, the IPCS gives the interval 2 – 18% by weight for alkali metal oxides and alkaline earth metals, but adds that there are fine special-purpose glass fibers exceeding this 18% limit. The Chemicals Inspectorate's classification of synthetic inorganic fibers is used in Appendix 1.

There are several *crystalline inorganic synthetic fibers*, but the only one taken up in this document is silicon carbide (SiC), since the question of its possible toxicity has received particular attention. Silicon carbide, which can occur in both particle and fiber form, is produced by heating quartz sand, coke, graphite, sawdust and rice hulls in electric crucibles. The fibrous form of silicon carbide can occur as *whiskers* – extremely fine monocrystalline fibers in the same size range as asbestos fibers, which moreover are virtually insoluble. Silicon carbide is marketed under such names as Crystalon, Carborundum, Carbonite etc.

Fiber characteristics and fiber definitions

Biological activity

Length and diameter, which determine a fiber's ability to penetrate respiratory organs and lung tissue, and resistance, or its ability to remain in lung tissue without being dissolved or carried away by the defense systems of the lungs (biopersistence), are generally regarded as the most important factors in assessing a fiber's toxic properties.

It is well known that fibers with a diameter below 3 μm are respirable, i.e. can be deposited in the lungs. Respirable fibers having a length greater than 8 μm and high biopersistence are regarded as having high biological activity and conse-

quently toxicity. However, it should be borne in mind that the toxicity and biological effects of a fiber are not simply a matter of the fiber's diameter and length. Characteristics such as chemical composition, surface activity, and substances or particles that are transported by the fibers into the respiratory passages can also be relevant. Unlike asbestos, the synthetic vitreous fibers do not split longitudinally with handling: i.e. they do not break into thinner fibers, though they may break into shorter ones. Mineral wool products normally produce less dust than asbestos, primarily because they are treated with mineral oil to contain dust but also because of the structural difference between asbestos fibers and mineral wool fibers. The airborne mineral wool fibers generated during handling, however, are mostly respirable.

Lung tissue can most easily cope with fibers that are shorter than 5 μm. When the fibers exceed 10 μm in length, it is difficult for the macrophages in the alveoli to envelop them (phagocytosis) and carry them away to be removed from the respiratory passages via ciliary clearance. Fibers longer than 15 – 20 μm, once deposited in the lungs, can therefore remain in lung tissue for a long time. If they break into shorter particles they become more susceptible to being either carried away or dissolved by the body's defense mechanisms (33, 34, 53, 65).

A further factor determining the fiber exposure of the lungs is the solubility of the fiber in the body. Long fibers that are physically and chemically resistant remain in lung tissue for a long time once they have been deposited there. The more soluble a fiber is in body fluids, the shorter should be its stay in the lungs. Long, thin fibers not dissolved by contact with body fluids tend to remain in the lungs for a long time after being deposited, which increases the likelihood of toxic effects on lung tissue and respiratory passages. In general, synthetic inorganic fibers are 10 to 1000 times less persistent in body tissues than fibers of asbestos.

Experiments made with rats to quantify the biopersistence of fibers (> 20 μm) of various materials have yielded the following ranking, which roughly indicates biopersistence in body fluids. The comparison with asbestos (crocidolite and amosite) is an indicator of the fiber's ability to remain in lung tissue. The most biopersistent materials are first on the list (35):

> Asbestos (crocidolite) > asbestos (amosite) > refractory ceramic fibers, special-purpose fibers (E glass and 475 glass), rock wool (traditional) > glass wool > slag wool > rock wool (new type).

No specific comparison studies have been made with silicon carbide fibers (whiskers), but these fibers should probably be placed toward the left in the above ranking (2). In a study in which experimental conditions were not directly comparable, silicon carbide whiskers had greater biopersistence in rat lung than amosite (17).

Solubility of synthetic inorganic fibers in aqueous solutions composed to resemble body fluids has been given considerable attention during recent years, ever since it was found that solubility in such liquids (solubility *in vitro*) can be

used to estimate biopersistence. In the comparative studies that have been made, the biopersistence of a fiber is closely related to its solubility (37).

Fiber definitions

Length and diameter are the most important physical characteristics in a fiber's description. These parameters are the basis for the fiber definition most commonly used in an occupational health context: a particle that is at least three times as long as it is wide, or an *aspect ratio* equal to or greater than 3:1. This was initially a practical limit for distinguishing a "fiber" from an irregular particle under a microscope with an acceptable degree of accuracy. To be considered in the context of occupational exposure limits, a fiber has to be respirable: it has to be able to reach the lungs and be deposited there after inhalation. The fiber diameter can therefore not exceed 3 μm. Further, a respirable fiber must be at least 5 μm long to be included in estimates of hazardous fibers. These fiber criteria are associated with fiber counts using an optical microscope.

In 1990, a different aspect ratio was introduced in Sweden: at least 5:1 (4). The reasons given for this were the following (73);

1. The Board of Occupational Health shares the general medical opinion that it is the long, thin fibers that constitute the greatest risk for tumor formation.
2. Unlike asbestos, the synthetic inorganic fiber materials that are covered by regulations tend to break rather than split during handling, thus creating shorter fibers.
3. A new exposure limit was being planned for natural crystalline fibers other than asbestos, and particle fragments in the aspect ratio interval 3:1 – 5:1 were looked on as a potentially large source of error.
4. With reference primarily to experience with asbestos, it was argued that a change of the criterion from 3:1 to 5:1 would mean a rise in the exposure limit in theory, but not in practice.
5. It was considered more important to establish an exposure limit for the natural crystalline fibers than to wait until the consequences of keeping the 3:1 limit were known in detail.

To strengthen the argument, there was a small study in which dust samples of synthetic inorganic fibers were counted using the two different criteria (73). The fiber count was about 15% lower when the 5:1 criterion was used. This difference was supported by a later study of refractory ceramic fibers (51) in which fiber counts for persons who handled the fiber material (and were thus directly exposed to the fibers) were about 20% lower when the 5:1 criterion was used. For indirectly exposed persons (those who did not handle the material but worked in the same building) the spread was too large to allow a correlation to be shown. The reason given was interference from particle fragments in background dust, which had a particularly large effect on the result when the 3:1 criterion was used.

In general, it can be said that there are compelling reasons to use an aspect ratio greater than 3:1 when counting actual fibers through an optical microscope. Dr. Henry Walton, who introduced the 3:1 criterion, has since become one of the

driving forces behind the movement to change it. The main reason has been the subsequent discovery that there is often considerable risk that particle fragments are counted as fibers. In rebuttal it was often argued that nobody knows whether the fiber-like particles in the 3:1 – 5:1 interval are hazardous to health. The American unions opposed a change of the original fiber criterion, primarily because it would mean a loss of continuity in the long history of monitoring asbestos dust in the U.S. Largely due to this, international efforts to launch the 5:1 criterion stopped within the ISO (45). For ambient air, however, the ISO published a standard in 1995 for determining asbestos fibers by transmission electron microscopy (TEM) (46), in which the 5:1 criterion was used as the fiber definition.

The task of establishing universal fiber criteria was continued by WHO, which in 1997 published its conclusion that fibers, both natural and synthetic, that should be considered in a health context should have aspect ratios greater than 3:1, diameters below 3 μm, and lengths greater than 5 μm (86). The EU also uses this fiber definition. Despite the strong arguments in favor of introducing the 5:1 criterion, WHO's final pronouncement of what should be regarded as a fiber in the context of health should be given precedence. This and the increasing (and increasingly easy) international exchange of research information – especially in the field of occupational hygiene – provide reason enough to recommend that Sweden adopt the WHO fiber definition. Further, during 2002 the ISO published two monitoring standards, one for determining inorganic fibers in ambient air by scanning electron microscopy (47) and one for determining air quality by phase contrast optical microscopy (45), in both of which the fiber criterion > 3:1 is used.

The only practical problem with return to the 3:1 criterion for fibers is that the laboratories now making fiber counts must be informed of the change. Regarding exposure limits, attention should be paid to the approximately 20% difference in fiber counts known to be obtained with the 3:1 versus the 5:1 criterion.

Nominal fiber diameters

The processes used to produce synthetic inorganic fibers may give rise to inconsistencies in fiber length and diameter. To describe their fiber products the manufacturers use the term *nominal diameter*, which is a length-weighted average diameter. The nominal diameters for continuous fiberglass products range from 3 to 25 μm; for most of them the nominal diameter is about 6 μm or a little more. Products of mineral wool, such as glass and rock wool, generally have nominal diameters of 4 to 5 μm. Refractory ceramic fibers are generally produced with nominal diameters in the range 1.2 – 3 μm. Special-purpose glass fibers are produced with nominal diameters of 0.1 to 3 μm. Production processes for non-continuous fiberglass products, however, tend to generate fiber diameters with a considerable spread around the average value. The final product will thus contain fibers both much thinner and much thicker than the average. All mineral wool products contain respirable fibers. There is no information on a nominal diameter for silicon carbide whiskers, but the average diameters and lengths of industrial

SiC whiskers have been reported by Cheng *et al*. (12) and Johnson *et al*. (49). They give an average diameter below 1 μm and average lengths ranging from about 4 to 20 μm.

Monitoring methods for fiber exposure

Occupational exposure to fibers occurs during fiber production, processing and use, and in connection with installation, replacement and demolition of materials and products containing fibers. Airborne fibers in work environments are monitored by sampling with personal monitors equipped with membrane filters (58). The filter is then made transparent, and the fibers are counted through a phase-contrast optical microscope. There is nevertheless a lower limit to what can be discerned through an optical microscope, and for fibers this limit is a diameter of about 0.2 μm. Fiber counting is a quantitative analysis based on the above-given criteria for respirable fibers. The count is based on shape alone, since fiber type cannot be identified by this method. It is instead assumed that the fibers observed are of the type being monitored – in this case synthetic inorganic fibers. WHO has published a detailed description of the methodology used for this type of fiber monitoring (86). If the fibers must be identified, a polarizing microscope or scanning electron microscope is used. A scanning electron microscope (SEM) equipped with an energy-dispersing x-ray spectrometer (EDS) must be used to identify fibers in air samples. WHO has also published a detailed description of this method (85). Scanning electron microscopy allows fibers > 0.1 μm, i.e. virtually all vitreous synthetic inorganic fibers, to be both seen and identified. SEM has also been used experimentally to determine the dimensions of SiC whiskers (10).

Effects on health

Health effects and test systems

The body responds to inhaled foreign material deposited on lung tissue (including fibers) with an inflammatory reaction. This is a normal defense mechanism and usually clears up rapidly. A long-lasting or even permanent reaction can occur if the fibers, due to their length or composition, resist the body's normal defense mechanisms. As long as the fibers remain in the lungs, the body will keep trying to get rid of them with the inflammatory reaction. Long-term or repeated inhalation of biologically aggressive fibers can result in a chronic reaction in respiratory passages or lung tissue, notably proliferation of connective tissue (fibrosis) or tumors (cancer in respiratory passages or lungs, pleural mesotheliomas). Several animal studies have been made to confirm the ability of various fibers to induce inflammation and fibrosis in respiratory organs. Exposure levels in these studies have been much higher than those known to cause symptoms of irritation in people, and the studies are therefore taken up in this report only in exceptional cases. Although they confirm that these fibers are able to produce inflammation

and irritation in the respiratory passages, dose-effect relationships are difficult to assess in relevant terms. Skin exposure to fibers can also cause local irritation.

Pulmonary fibrosis

One type of tissue reaction is pulmonary fibrosis. Inhalation and deposition of fibers and the repair processes triggered by contact with foreign material, if continued long enough, can lead to destruction of tissue cells and transformation of connective tissue. A connective tissue change of this sort is a pathological change, and in advanced stages it is visible on x-rays. It is usually possible to measure the reduction in lung function caused by fibrosis before the changes are severe enough to show up on ordinary x-rays.

Lung cancer

Prolonged inhalation exposure to asbestos fibers can increase the risk of lung cancer, particularly bronchial cancer (cancer in the epithelium of the respiratory passages). The mechanisms are not known. Some of the available scientific documentation indicates a connection between development of scar tissue (fibrosis) in the lungs and cell transformation (tumor initiation) in the affected tissue. Other data suggest the possibility that asbestos fibers have a direct effect on the nuclei of cells in the bronchial epithelium (a genotoxic effect). It has also been proposed that the fibers are carriers of carcinogenic substances. Several studies have shown a strong synergistic effect between exposure to asbestos fibers and smoking in the occurrence of lung cancer. Asbestos fibers are mentioned here as a good example of biologically aggressive fibers. Some kinds of synthetic inorganic fibers resemble asbestos in one or more ways (e.g. biopersistence), which should be taken into consideration in estimating their biological effects.

Mesothelioma

Mesotheliomas are malignant tumors in the pleural or peritoneal mesothelium (the lining of the lung or body cavity). They have been observed in people after exposure to several kinds of asbestos fiber. They have also been induced in laboratory animals by inhalation of mineral fibers other than asbestos and by some kinds of synthetic inorganic fibers. The mechanism by which fibers cause mesotheliomas is unknown. The fibers are probably transported from the deposition site in the lungs into direct contact with the mesothelium, where they can initiate a cell transformation to malignant tumor tissue – either via development of fibrosis or via the same kind of genotoxic effect they have on lung tissue. The tendency of some kinds of fibers to cause mesothelioma has been exploited in cancer research and in the design of test systems for studying the carcinogenicity of fibers. The experimental exposure to fibers is via inhalation, intratracheal instillation, or direct injection into the pleural or peritoneal cavity.

Test systems

In general for all test systems, the fibers should be closely defined as to type(s), length and diameter.

In assessing animal experiments, the exposure methods used should be examined. It is now generally accepted that *in vivo* studies based on intrapleural or intraperitoneal injection are less specific and thus less relevant to risk assessments than studies in which the animals inhale the fibers. The injection studies do not reflect natural exposure conditions and the doses are high, yielding non-specific results that are difficult to interpret. Moreover, the pleural and peritoneal membranes lack the mechanisms for removal of foreign material that are found in the lungs and respiratory passages. The relevance of these studies, and interpretation of the results in terms of human exposures at the workplace, have therefore been questioned. In any case, these results should be assessed in an overall perspective that includes results from other types of tests.

In vitro tests – tests made on cultures of mammalian cells or tissues – have the disadvantage of being less specific and thus more difficult to interpret in relevant terms.

The tests specified by the European Commission reflect the prevailing mechanistic approach, which was summarized in an EC publication in 1999 (26). This document describes test systems for:

- biopersistence with short-term inhalation exposure
- biopersistence with intratracheal instillation
- carcinogenic effect with intraperitoneal injection (rats)
- chronic inhalation (rats)
- sub-chronic inhalation tests for toxicity

Effects on skin

Synthetic inorganic fibers can cause itching and irritation simply by getting on the skin. Most of this mechanical irritation is probably caused by fibers greater than 5 μm in diameter (28, 32, 70). This irritation can cause or exacerbate eczema, especially in persons who have or have had atopic dermatitis. These people are unusually susceptible to the skin rashes from irritation by synthetic inorganic fibers (78). Dermatitis caused by synthetic inorganic fibers is a common clinical and occupational health problem, and can be quite severe for atopics if they do insulation, construction or electrical work (29, 78). In Sweden, about 20% of young people today have atopic dermatitis (7).

Allergic contact eczema caused by allergenic substances (usually epoxy and phenol-formaldehyde resins) used to bond or coat fiber products has been reported in association with both production and use (29, 78).

Continuous glass fibers

Continuous glass fibers in general have been found to have a low degree of toxicity to both humans and experimental animals. It should be borne in mind that most of these fibers are not respirable because they are more than 3 μm in diameter (usually over 6 μm).

Human data

Epidemiological studies of occupational groups exposed to continuous glass fibers and fibers from insulation wool have revealed no correlation between the exposures and development of chronic lung disease. Nor has it been possible to show an increase in risk of lung cancer or pleural or peritoneal mesothelioma that can be definitely attributed to exposure to continuous glass fibers. The studies have covered large groups occupationally exposed to these fibers. In the assessment of the IARC, it can not be determined on the basis of present knowledge whether these fibers are carcinogenic (42),

Animal data

No inhalation studies have been made with laboratory animals. There are a number of animal studies in which glass fibers were injected into the pleural cavities of rats and some studies with peritoneal injection into laboratory animals. The results were negative: there was no difference in tumor incidence between treated animals and controls. (These studies are reviewed in Reference 42.)

Fibers of glass wool, rock wool and slag wool

Human data

There have been epidemiological studies exploring the possibility of a connection between mineral wool exposure and lung fibrosis, changes in pleura, chronic bronchitis, chronic obstructive lung disease, emphysema and asthma. No convincing evidence of any such connection was found (18, 42). One study reported an elevated risk for obstructive lung disease among workers exposed to mineral wool if they were also heavy smokers (31).

Exposure to mineral wool has been connected to irritation of eyes and upper respiratory passages (coughing, nasal congestion, smarting/burning eyes, nose or throat) in construction workers who did insulation work. The symptoms increased in proportion to the number of hours per month they worked with the material (68). No direct exposure measurements were made in this study, but the exposure level around this kind of work has been reported to be ≤ 3 fibers/ml (76).

A similar Swedish study (3) is based on medical examinations given to construction workers throughout the country at intervals of two to three years. The cohort included men born in 1955 or later who had been given spirometry tests during the 1984 – 1993 period and for whom both job title and self-reported exposure information were available (n= 83,993). For the 1989 – 1992 period, respiratory symptoms were also registered (n= 45,716). Results from at least two spirometry tests taken in 1984 – 1993, job title, and exposure information given by the subjects themselves were available for 20,086 men. Exposures were classified by combining an occupational hygienist's estimate based on job title (job-exposure matrix) and the descriptions of exposure given by the subjects at their physical exams. The analysis models included, in addition to mineral wool, exposure to quartz, asbestos and isocyanates, as well as smoking habits. An internal reference material was used for spirometry. No relationships between

exposure to insulation wool and VC or FEV_1 were seen in either a cross-sectional analysis (n = 83,993) or a longitudinal analysis (n = 20,086). There was, however, an elevated occurrence (Prevalence Odds Ratio 2.6; 95% CI 2.2 – 3.6) of persistent cough reported at the 1989 - 1992 physical exams by those workers classified as highly exposed to insulation wool (n = 1747). The requirements for this classification were both a job title which according to the job-exposure matrix indicated work with the material (insulation installer, pipelayer, carpenter) and the subjects'own statements that they had worked with the material for at least 2 hours per day during the previous year. Comparisons were made with construction workers classified by the same method as unexposed (n = 4280). In the analysis, adjustments were made for exposure to asbestos and quartz and for smoking habits, all of which also yielded independent and statististically significant risk increases. The estimated effect of this high exposure to insulation wool was comparable to that of smoking (POR 2.6; 95% CI 2.4 – 2.8). For groups with lower exposure to insulation wool there were smaller but still significant elevations in risk. Samples of respirable fibers (n = 125), taken with personal monitors during the 1978 – 1990 period and analyzed by the same occupational health organization, showed median contents of 0.10 – 0.42 fibers/ml air (75th percentiles 0.19 – 0.74 f/ml) for insulation installers and carpenters (not available for pipelayers). Total dust measurements (n = 42) were available only up to 1985, and showed median contents of 0.98 – 3.15 mg/m^3 (75th percentiles 1.00 – 12.9 mg/m^3).

Since the early 1970s, numerous large-scale epidemiological studies have been made to determine whether exposure to synthetic inorganic fibers – especially fibers of rock, glass and slag wool – increases the risk of cancer, particularly lung cancer and mesothelioma.

In nearly a dozen cohort studies, several of which were supplemented by case-control studies within the cohort, elevated mortality due to lung cancer was observed in workers in mineral wool production industries. (A systematic review of these studies is found in Reference 5.) The Standardized Mortality Ratio (SMR) for glass wool fibers ranged from 0.82 to 1.99. For rock wool fibers, SMRs ranging from 1.22 to 1.53 are reported in three studies (5). In an analysis of the data collected in these studies it was not possible to definitely connect the elevations in risk to just these fibers (42). None of these studies revealed a dose-response relationship between exposure to the studied fibers and risk of death due to lung cancer. In addition, there are uncertainties in the calculation of SMR, where national mortality figures were used for comparison without adjustment for regional variations or socioeconomic differences (including smoking habits) (5, 6). Further factors to consider in assessing the studies are exposure to other carcinogenic substances such as PAH and arsenic, either along with the mineral wool fiber or independently. Another difficulty is arriving at a satisfactory assessment of previous exposures, although asbestos probably did not contribute to the observed risk increases. The studied groups were chosen to minimize the risk that exposure to asbestos would be a confounding factor in the analyses. No elevation in mesotheliomas was observed in these studies, which supports the

assumption that asbestos exposure can be eliminated as an explanatory factor (5, 42).

It must be emphasized that in these studies the estimated exposures to fibers of glass wool, rock wool or slag wool were in general low compared to historical exposures – to asbestos, for instance – and that any increase in cancer risk can therefore be difficult to show. For example, one estimate for rock wool exposure was about 1 f/ml (13, 23), interval 0.03 – 1.42 f/ml (23). Exposure to asbestos at 1 f/ml for 30 years has been estimated to yield a SMR of 1.3 (5, 19).

Animal data – fibers of glass wool and rock wool

Studies in which laboratory animals were exposed by inhalation to fibers of glass wool or rock wool have shown no effects on tissues or organs in the form of tumors, and glass wool fibers did not cause lung fibrosis. A few animal studies made with rock wool fibers showed some changes in connective tissue (42).

Tumor formation in the pleural or peritoneal membrane has been observed in a large number of studies after direct injection or implantation of fibers of glass or rock wool. As mentioned previously, however, this type of study is now considered to be an insufficient basis for assuming that inhaling the fibers could cause tumors. The studies are mentioned here only because they were previously considered quite important. (See for example the assessment of man-made mineral fibers published in 1988 by the IARC: Reference 41).

Special-purpose glass fibers

Human data

There is no epidemiological material in the form of published scientific reports that can be used for assessing effects on human health.

Animal data

For one kind of special-purpose vitreous microfiber produced at high temperatures, E glass and 475 glass, there is an inhalation study with rats. In the rats (38 animals) exposed to 475 glass fibers there were 4 lung adenomas, which the authors regarded as not significantly different from controls (38 animals, 1 carcinoma and 1 adenoma). In the rats exposed to E glass fibers (43 animals) there were 7 carcinomas and 3 adenomas in lungs, and 2 mesotheliomas (16). There is also a study in which hamsters were exposed to 475 glass: one case (1/83) of mesothelioma was observed in the exposed group (36, 65). Occurrence of lung tumors and mesotheliomas has also been reported in studies in which the animals were exposed to the fibers by intratracheal instillation (71, 72).

Refractory ceramic fibers (RCF)

Production and industrial use of refractory ceramic fibers have been steadily increasing since the beginning of the 1970s. Since RCF materials liberate fibers of respirable dimensions when they are processed, and since these fibers are relatively insoluble in body fluids and have high biopersistence, exposure to RCF should be regarded as potentially coupled with higher risk than exposure to

other kinds of synthetic inorganic fibers. When RCF material is used at high temperature it may be transformed into crystalline forms (silica, crystalline silicates and cristobalite; described above under Classification of synthetic inorganic fibers). Samples of RCF that has been heated can contain up to 37% cristobalite (8), but the variation is considerable and samples of used furnace linings with little or no cristobalite content have been reported (51). Air samples from around removal of used furnace linings have contained 4 to 15 % cristobalite when the furnace lining had been exposed to temperatures in the range 500 – 2550 °C for 130 to 471 hours (30, 61), and 75% of the samples taken with personal monitors reached or exceeded the exposure limit for cristobalite (30). Crystallization does not mean that fibers lose their fibrous shape, but the heating probably makes them more brittle and thus more likely to break during handling and produce more dust with shorter fibers (51). If the dust released during removal of the furnace linings contains cristobalite it constitutes a potential risk for silicosis.

Exposure data from RCF production and use are presented in Appendix 3.

Human data

No epidemiological studies of cancer risk in groups exposed to refractory ceramic fibers have yet been published.

As to effects other than cancer, the IARC summary published in 2002 mentions reports of pleural plaque (thickening of the pleural membrane) in workers exposed to RCF for 20 years or more (55, 59). In a study made by Lockey *et al*. a correlation was observed between estimated cumulative exposure to RCF (the number of months of exposure x the content of RCF fibers in inhaled air = fiber months, or fm) and the occurrence of changes in pleura, primarily pleural plaque. Significantly higher odds ratios were seen in the groups with the highest (> 135 fm/ml; OR 6.0; 95% CI 1.4 – 31.0) and the next highest (> 45 – 135 fm/ml; OR 5.6; 95% CI 1.5 – 28.1) cumulative exposures, when they were compared with the lowest exposure group (> 0 – 15 fm/ml). This correlation was seen after control for previous asbestos exposure. After a latency time of > 20 years, 8% of exposed subjects had pleural changes (59). In the group with the highest cumulative dose (> 135 fm/ml) there was also a (not significant) correlation to irregular opacities seen on lung x-rays (OR 4.7; 95% CI 0.97 – 23.5, adjusted for age, tobacco consumption etc.).

A study by Cowie *et al*. (15) of 774 RCF-exposed workers revealed an elevated incidence of respiratory symptoms (particularly bronchitis) with some indication of a relationship to increasing cumulative exposure over time. The authors' interpretation of this observation was that the symptoms were due to local irritation of respiratory mucous membranes by the fibers. This study also reports slightly elevated occurrences of pleural changes and pleural plaque related to the time since initial exposure, which on average was only 9.6 years. Of the 355 exposed workers who had not previously been exposed to asbestos, only 9 had pleural plaque. A slightly elevated occurrence of changes visible on x-rays (small opacities in lung tissue) was also reported. These changes, however, could not be

correlated to the amount of RCF exposure, and the authors report the observation as uncertain. Among male smokers in the studied population there was (after adjustment for tobacco consumption) a reduction in lung function (FEV_1 and FVC) that correlated to cumulative exposure to refractory ceramic fibers. The reduction was about 20 ml for FEV_1 and FVC per fiber-year/ml. No effect was seen in non-smokers. The authors' interpretation of this observation was that a slight functional reduction of the restrictive type may occur as a combination effect of exposure to RCF and tobacco smoking.

Employees in a company producing RCF (production workers as well as other employees; 592 men and 144 women) were given spirometry tests. Time-weighted median exposures ranged from 0.01 to 1.0 fibers/ml and median exposure times were 8.5 years for men and 4.0 years for women. After adjustment for factors such as tobacco consumption and age-related changes, male smokers and ex-smokers showed a significant further reduction of FVC (165 ml and 155 ml respectively with 10 years of exposure) and the male smokers also had a significantly lower FEV_1 (135 ml/10 years). Non-smoking women had a significantly lower FVC (350 ml/10 years) (56). According to the authors, the observed differences between men and women may be due to differences in exposure time, or may reflect a sex difference in sensitivity, or may be because the group of women was small (56).

Dry cough and eye irritation were more common in workers exposed to RCF concentrations of 0.2 – 0.6 f/ml than among workers with lower exposures. The odds ratio for dry cough was 2.5 (95% CI 1.3 – 5.1) and for eye irritation 2.2 (95% CI 1.3 – 3.5) (81). There were 628 participants in this study, 617 (98%) of whom were exposed to concentrations below 1 f/ml. In this study also there was a correlation between cumulative exposure to RCF and reduction of FEV_1 in both smokers and former smokers. The authors concluded that cumulative exposure to ceramic fibers can result in obstructive reduction of lung function by amplifying the effect of smoking (81).

LeMasters *et al*. (57) report specific causes of death for men employed at two companies producing RCF. Mortality in this group was lower than that in the general population, possibly indicating a healthy worker effect. Mortality due to lung cancer and non-malignant lung diseases was not elevated. No cases of mesothelioma were observed. The strengths of this study are a reasonable latency time (median 23 years) and detailed histories of occupation and smoking habits based on interviews with most of the subjects. Its weaknesses are the above-mentioned healthy worker effect and the relatively small size and low age of the cohort (median age 50) at the end of the follow-up period. The statistical power for discovering, for example, a doubled risk of lung cancer was only 40%, which greatly limits the study's information value. An elevated mortality due to cancer or malignant tumors in the urinary system was seen. This observation was based on only a few cases (5 observed; 1.45 expected) and the authors draw no conclusions from it regarding a causative relationship between exposure to RCF and urinary cancer. They point out that the observation was not based on any advance

hypothesis and that a credible biological mechanism for such damage has not yet been proposed.

Animal data

In well-designed inhalation experiments, rats that inhaled RCF developed lung fibrosis and had an elevated incidence of lung tumors (adenomas). Similar tests made with hamsters have resulted in mesotheliomas. There are also other studies in which no such results were seen. In a long-term study in which rats were exposed by inhalation to several types of RCF (62), both lung fibrosis and several cases of mesothelioma were observed. See also Hesterberg and Hart 2001 (37). In long-term studies with rats and hamsters, RCF has also been found to yield pleural fibrosis (62, 64).

Summary comments on refractory ceramic fibers

So far, we have only about 30 years of experience of exposure to RCF. This leaves the possibility that a risk of mesothelioma, and also lung cancer, may exist, but with a latency time that will allow it to be observed only in future epidemiological studies. There are animal studies that support the proposition that refractory ceramic fibers are carcinogenic and that inhaling them results in pleural fibrosis.

Refractory ceramic fibers irritate eyes and respiratory passages. There are also studies indicating that occupational exposure to RCF affects lung function, but it has not been determined whether the effect is restrictive or obstructive in nature. The estimated effect of exposure on lung function was (per fiber-year/ml) of the same magnitude as one pack-year of tobacco consumption, which indicates that it may be clinically relevant. There are two studies in which exposure to RCF is associated with development of changes in pleura, primarily pleural plaque. One of these studies included a control for previous exposure to asbestos, and in the other study the RCF-exposed workers were judged to have been previously unexposed to asbestos. However, it is always difficult to estimate prior exposure to asbestos.

Silicon carbide (SiC)

Silicon carbide is a hard, synthetically produced crystalline compound of silicon and carbon. It has been used since the 19th century in sandpaper and for cutting and polishing, and more recently as heat insulation in the semiconductor industry. Particles of silicon carbide may be coated with amorphous or crystalline silicon dioxide.

Silicon carbide occurs as amorphous dust, irregularly shaped particles and crystalline fibers (whiskers). Bye *et al*. (10) report that 80% of the airborne fibers resulting from industrial production of silicon carbide are <0.5 μm in diameter and >5 μm in length. Since they are below 3 μm in diameter they are respirable.

Silicon carbide in non-fibrous form has very low toxicity to both humans and laboratory animals (9, 67).

Silicon carbide in fibrous form can generate respirable fibers with demonstrated biological activity similar to that of asbestos fibers. Inhaled fibers of silicon carbide have been found to have high biopersistence, meaning that they can remain in lung tissue for a long time (77). There are also case reports of pulmonary fibrosis attributed to silicon carbide. A specific form of pneumoconiosis and reduced lung function have been observed among employees exposed to silicon carbide in production of Carborundum.

Human data

For epidemiological studies of exposure to silicon carbide, whether to fibers or non-fibrous particles, assessment and interpretation of results are made more difficult by the fact that the exposures are usually mixed and include other potentially toxic air pollutants. In a work by Dufresne *et al*. from 1993 (20) it is reported that examination of the fiber content in the lung tissue of a SiC-exposed worker revealed that about 30% of the fibers were SiC. The others were amorphous or crystalline quartz mixed with silicon and iron, and silicon-containing fibers from kaolinite (20).

The following have been observed in clinical and epidemiological studies:

- nodular changes in lungs similar in appearance to those of silicosis
- connective tissue changes associated with the nodular changes
- reduction in lung function
- asthma, emphysema and chronic bronchitis

These observations are reported by Dufresne *et al*. (20, 21), Durand *et al*. (22), Osterman *et al*. (66), Romundstad *et al*. (75) and others. For a review of the literature see Vaughan *et al*. (83). These studies were made in silicon carbide production facilities and the exposures were complex, making it impossible to definitely connect exposure to silicon carbide fibers – as distinct from other dust particles – to effects on the lungs and draw any conclusions on dose-effect or dose-response relationships.

Evidence of elevated risk of lung cancer associated with production of silicon carbide has been reported in two epidemiological studies.

In 1994, Infante-Rivard *et al*. (43) made a retrospective cohort study of silicon carbide production workers in Quebec, Canada. They identified an elevated risk of mortality due to respiratory diseases other than cancer (SMR 2.0; 95% CI 1.2 – 3.2) as well as lung cancer (SMR 1.7; 95% CI 1.1 – 2.5). Lung cancer mortality increased weakly with cumulative exposure to total dust. However, it should be remembered here that the exposure to silicon carbide was combined with exposure to other air pollutants and that only total dust was measured.

A Norwegian study reported by Romundstad *et al*. (74) covered 2,620 men exposed to silicon carbide. In addition to silicon carbide fibers, the exposure consisted of particulate silicon carbide and crystalline silicon dioxide. There were strong correlations between all these exposures and between each of them and lung cancer. In analysis of the correlation between exposure and development of lung cancer, cumulative content of silicon carbide fibers showed a dose-response relationship but crystalline silicon dioxide did not, when both exposures were

included. Elevated risk of lung cancer was also seen in the lowest exposure group (0.1 – 0.9 fiber-years/ml). However, exposure to silicon carbide fibers could not be differentiated from exposure to particulate silicon carbide. There was an elevated risk of lung cancer for the entire cohort: a SIR (Standardized Incidence Ratio) of 1.9 (95% CI 1.5 – 2.3) (74). As in other studies, this group of industrial workers had been simultaneously exposed to other air pollutants: particulate silicon carbide, sulfur dioxide, crystalline silicon dioxide (including cristobalite) and low levels of polyaromatic hydrocarbons.

Animal data

The biological activity of silicon carbide fibers has been confirmed in animal experiments. It is summarized by Vaughan *et al*. (82) as macrophage death, connective tissue formation and chronic accumulation of inflammatory cells.

Silicon carbide fibers have been shown to cause tumors in laboratory animals: fibers injected directly into the pleural cavity resulted in mesotheliomas (79). Lapin *et al*. (54), in a 13-week inhalation study in which rats were exposed to three extremely high dose levels of silicon carbide whiskers (630, 1746 and 7276 f/ml, 6 hours/day, 5 days/week) reported effects in the form of:

- inflammatory reaction in the respiratory passages
- hyperplasia and adenomatosis in mucous membranes
- bronchiolitis-alveolitis with thickened walls
- focal fibroses in pleural tissue

and also dose-response relationships between exposure levels and these effects.

In a study with intratracheal instillation, Petran *et al*. (69) observed inflammatory reaction, destruction of normal lung structure, and lung fibrosis in guinea pigs after a single 50-mg dose of SiC fibers. SiC fibers instilled into the trachea have been found to cause an inflammatory reaction that increases with exposure time and is accompanied by formation of inflammatory granulomas, but not tumors (82). Similar changes have been observed after exposure to asbestos fibers (crocidolite). It should be observed that the doses used in these experiments were high, implying that the effects might also be ascribed to overloading the lungs with particles, including fibers. The results can not be unreservedly accepted as effects of the fibers alone.

Studies have also been made in which rats were given intrapleural inoculations of silicon carbide whiskers, resulting in mesotheliomas (49). Exposure of cell cultures to silicon carbide fibers (*in vitro* method) has yielded evidence of cytotoxic effects corresponding to those of amosite asbestos.

Summary comments on silicon carbide

It is clear that fibers of silicon carbide are biologically active. The critical organ for their effects appears to be lung tissue. Workers exposed to silicon carbide have silicosis-like changes in lungs, but their simultaneous exposure to other substances (including quartz) makes it impossible to definitely correlate these changes to their exposure to silicon carbide fibers. It is reported in two epidemiological studies that exposure to silicon carbide fibers during manufacture of products

containing silicon carbide is associated with an elevated risk of lung cancer. In both of these studies the workers were simultaneously exposed to other substances of a type that makes it impossible to definitely attribute the observation to the exposure to silicon carbide fibers.

Dose-response / dose-effect relationships

Symptoms of irritation in eyes and upper respiratory passages have been reported after exposure to mineral wool at estimated exposure levels of ≤ 3 f/ml (68). Persistent cough has been reported at a median exposure of 0.1 to 0.42 f/ml (75th percentiles ranged from 0.19 to 0.74 f/ml) to insulation wool (3).

A large amount of epidemiological data has been collected, but no correlation between lung cancer and exposure to mineral wool has been shown. Exposure levels in many of the studied populations have been low, however – around 1 fiber/ml or lower. The possibility of a cancer risk at higher exposures has not been examined. The IARC assessment (42) is the same.

Dry cough and eye irritation were more common among workers exposed to refractory ceramic fibers at levels of 0.2 to 0.6 f/ml than among workers with lower exposures (81).

Declines in lung function (FEV_1 and FVC) have been reported in male smokers with an average exposure to RFC levels of 0.9 – 2.1 f/ml, with an estimated reduction of about 100 ml per 5 fiber-years/ml for both values (15). Further, a reduction of FVC has been observed in male smokers and former smokers, and a reduction of FEV_1 in male smokers, after 8.5 years (median) of exposure to 0.01 – 1.0 f/ml. Non-smoking women showed a significant reduction of FVC at the same exposure level and a median exposure time of 4.0 years. There were only a few women in this study, however (56).

A significant increase in the number of pleural changes, primarily pleural plaque (indicating that the fibers had come into contact with the pleural membranes), was reported in RCF-exposed workers at a cumulative exposure of > 45 – 135 fm/ml, in comparison to a group with lower exposure (59). A further observation in this study was that lung changes visible on x-rays tended to be more frequent in the group with the highest cumulative dose (>135 fm/ml).

Silicosis-like changes have been observed in the lungs of workers exposed to silicon carbide, but the correlation to exposure to SiC fibers is not clear and no conclusions on a dose-effect or dose-response relationship can be drawn. Two epidemiological studies have shown a connection between exposure to silicon carbide and development of lung cancer (43, 74), but the degree to which silicon carbide fibers contributed to the elevation in risk is not clear. In the study by Romundstad *et al*. an elevated risk of lung cancer was found even in the lowest exposure group (0.1 – 0.9 fiber-years/ml) (74).

Conclusions

The critical effect of occupational exposure to **mineral wool fibers** is irritation of the respiratory passages (persistent cough). This has been observed at median exposure levels of 0.1 to 0.4 fibers/ml.

There are no data on which to base a critical effect of occupational exposure to **special-purpose glass fibers**. These fibers, including E glass and 475 glass, are considered to be potentially carcinogenic to humans.

The critical effect of occupational exposure to **refractory ceramic fibers** (RCF) is reduction in lung function. This has been observed in a small group of non-smoking women after 4 years (median) of exposure to 0.01 – 1.0 f/ml. At this exposure level there was also a reduction of FVC in male smokers and former smokers, and a reduction of FEV_1 in male smokers after a median 8.5 years of exposure. Irritation of eyes and respiratory passages has been observed at a minimum exposure level of 0.2 – 0.6 fibers/ml. There are indications that, for humans, long-term exposure to RCF can result in changes in pleura, primarily pleural plaque. RCF exposure has been shown to result in pleural fibrosis in laboratory animals. Data on biopersistence and the results of animal experiments indicate a carcinogenic potential, and RCF has been judged to be possibly carcinogenic to humans.

Studies of workers exposed to **silicon carbide fibers** are very difficult to interpret, since the exposures are always mixed and also contain crystalline quartz. Elevated occurrences of silicosis-like changes in lung tissue have been observed in these studies. An elevated risk of cancer has been correlated to many years of exposure to a level of 0.1 – 0.9 f/ml, but the exposure was mixed. Silicon carbide is regarded as possibly carcinogenic to humans.

For **synthetic inorganic fibers** in general, it can be stated that fibers more than 5 μm in diameter can cause itching, skin irritation and eczema (mechanical effects), especially in sensitive persons (atopics).

As to **fiber definitions**, Swedish criteria should be brought into line with the criteria established by WHO in 1997.

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Appendix 1. Definitions and applications of some of the words used on the subject of fibers.

Aerodynamic diameter: the diameter of a spherical particle with the density of 1 g/cm^3 which in still air or in air with laminar flow has the same rate of descent as the particle being described, whatever its actual size, shape or density.
Aspect ratio: the length:diameter ratio of a fiber.
Binder: a substance or compound that makes fibers in a product adhere to each other so that they can be shaped. Binders are usually phenol-formaldehyde or urea-formaldehyde resins.
Biopersistence: ability to remain in the body after being deposited there. A fiber's biopersistence in the lungs is determined by its solubility and by the lungs' ability to remove it.
Continuous fibers: Glass fibers of a specified diameter, usually exceeding 6 μm, drawn from molten glass through a nozzle.
Crystalline fibers: fibers with atoms arranged in a network/crystal lattice.
Fiber: a particle with an aspect ratio greater than 3:1 (WHO definition).
Microfiber: a fiber, usually a special-purpose glass fiber, with a diameter below 1 μm. Also called *superthin fiber*.
Mineral wool fibers: synthetic vitreous silicate fibers made from melted glass, rock or slag, in which the total content of alkali metal oxides and alkaline earth metals exceeds 18%. Fibers in mineral wool products are randomly oriented.
MMMF: man-made mineral fiber.
MMVF: man-made vitreous fiber, a sub-category of MMMF comprising the non-crystalline fibers. It includes fiberglass and insulation wools (glass wool, rock wool, slag wool).
Nominal diameter: a length-weighted average diameter of the fibers in a product composed of mineral wool.
Refractory: capable of enduring high temperature.
Respirable fiber: a fiber with a diameter of < 3 μm (as defined by WHO); to be included in a fiber count it must also be longer than 5 μm.
Silicate fibers: fibers consisting of compounds of Si, O and one or more metals.
SMF: synthetic mineral fibers.
Special-purpose fibers: usually fine, synthetic silicate fibers made from melted glass, in which the total content of alkali metal oxides and alkaline earth metals can range from 2% to more than 18%.
Refractory ceramic fibers; synthetic vitreous fibers produced from molten aluminosilicates, in which the total content of alkali metal oxides and alkaline earth metals is 18% or less.
Vitreous fiber: non-crystalline fiber.
Vitreous synthetic inoganic fibers: non-crystalline synthetic fibers produced from silicates.
Whiskers: inorganic monocrystalline fibers.
WHO fibers: All fibers with a diameter less than 3 μm, length at least 5 μm, and aspect ratio greater than 3:1.

Appendix 2. International classifications.

European Union

It is worth mentioning that the classification of mineral fibers has long been a source of controversy between the member nations of the EU (formerly the EEC). The Germans have long argued that fibers are in principle carcinogenic but may be exempted from this classification because of:

- chemical composition and a carcinogenicity index calculated on this basis
- the results of animal experiments in which the fibers are injected into pleural or peritoneal cavities
- the results of animal experiments with long-term inhalation or bronchial instillation of the fibers

In November of 1997 the European Commission established criteria for classification and risk labeling of synthetic inorganic fibers, based on assessments of possible effects on health. The document is the 23rd revision (97/69/EC) of the European Commission's Dangerous Substances Directive (67/548/EEC). In the EC system, all mineral wools of synthetic mineral fibers are classified as locally irritating and can in addition be classified as carcinogens in accordance with the Commission's separate system for this. The EC system for classification of carcinogens is partly, but not entirely, the same as the IARC system. The EC categories are:

1. carcinogenic
2. probably carcinogenic
3. possibly carcinogenic

According to the rules of the European Commission, fibers of mineral wool are in class 2 or 3 above, depending on their chemical composition and the results of solubility tests performed according to EC guidelines (26) for:

- biopersistence of mineral fibers – inhalation
- biopersistence of mineral fibers – intratracheal instillation
- carcinogenicity tests – intraperitoneal injection (rats)
- chronic toxicity test (rats) – inhalation of mineral fibers (rats)
- subchronic toxicity test (rats) – inhalation of mineral fibers (rats)

It should be observed that these test methods are not part of the Dangerous Substances Directive 67/548/EEC. They have the status of "methods recommended by the European Commission" but no legal status in the EU member countries unless they have been granted such by national legislation.

See also the pro memoria (ILSI Workshop Report) from the European Commission's Scientific Committee on Occupational Threshold Limits 2001 (27).

IPCS 1987

The International Programme for Chemical Safety (IPCS), run jointly by the World Health Organisation (WHO), the International Labour Organisation (ILO) and the United Nations Environmental Program (UNEP), produced a report in 1987 (44) containing appraisals of the health effects of mineral fibers. The IPCS

paid special attention to the local irritation of skin and mucous membranes of respiratory passages. Regarding carcinogenicity, the IPCS limited itself to observing that certain mineral fibers might cause lung cancer and recommending the use of personal protective equipment in situations involving occupational exposure to high air concentrations.

IARC 2002

In 1988, the International Agency for Research on Cancer (IARC) in Lyon, France (the cancer research institute of WHO), reviewed the available material on mineral fibers and on this basis made carcinogenicity assessments of them (41). At that time the IARC assessment for fibers of rock wool and slag wool was that there was 'limited evidence' that they caused cancer in humans, and fibers of glass, slag and rock wool were therefore placed in Group 2B - the substances were judged to be 'possibly carcinogenic to humans.' In November 2001 the IARC convened an expert group to make a comprehensive review of current and relevant scientific literature on the carcinogenic effects of mineral fibers and a new series of assessments.

The report containing these assessments has since been published (42). In the assessments made by the IARC in 2001, it was concluded, after examination of a large amount of scientific material, that only mineral fibers with high biopersistence should retain their classification as 'possibly carcinogenic to humans' (IARC Group 2B). This classification applies to refractory ceramic fiber (RCF) used industrially for insulation in high-temperature furnaces, as well as some special-purpose glass wool products not used for insulation. Fibers of the most commonly used mineral wool products, here meaning glass wool, rock wool and slag wool for insulation, no longer need be classified as carcinogenic to humans. They were judged by the IARC working group to belong in Group 3: "not classifiable with regard to carcinogenicity." The group placed continuous glass fibers in the same category.

In its report (42) the IARC summarized its findings as follows:

- There is inadequate evidence in humans for the carcinogenicity of glass wool.
- There is inadequate evidence in humans for the carcinogenicity of continuous glass filaments.
- There is inadequate evidence in humans for the carcinogenicity of rock (stone) wool/slag wool.
- There is inadequate evidence in humans for the carcinogenicity of refractory ceramic fibers.
- There is sufficient evidence in experimental animals for the carcinogenicity of special-purpose glass fibers including E-glass and '475' glass fibers.
- There is sufficient evidence in experimental animals for the carcinogenicity of refractory ceramic fibers.
- There is limited evidence in experimental animals for the carcinogenicity of insulation glass wool.

– There is limited evidence in experimental animals for the carcinogenicity of rock (stone) wool.
– There is limited evidence in experimental animals for the carcinogenicity of slag wool.
– There is limited evidence in experimental animals for the carcinogenicity of certain newly developed, more biopersistent fibers including fiber H.
– There is inadequate evidence in experimental animals for the carcinogenicity of continuous glass filaments.
– There is inadequate evidence in experimental animals for the carcinogenicity of certain newly developed, less biopersistent fibers including the alkaline earth silicate (X-607) wool, the high-alumina, low-silica (HT) wool and fibers A, C, F and G.

The following system has been used by the IARC to classify substances with regard to carcinogenicity:

Group 1: The agent (mixture) is carcinogenic to humans
Group 2A: The agent (mixture) is probably carcinogenic to humans
Group 2B: The agent (mixture) is possibly carcinogenic to humans
Group 3: The agent (mixture) is not classifiable as to its carcinogenicity to humans
Group 4: The agent (mixture) is probably not carcinogenic to humans

and the overall assessments of the IARC for man-made vitreous fibers, after weighing all the evidence from studies of humans and laboratory animals, are:

– special-purpose glass fibers such as E glass and 475 glass are possibly carcinogenic to humans: IARC classification Group 2B.
– Refractory ceramic fibers are possibly carcinogenic to humans: IARC classification Group 2B.
– Fibers from insulation material of glass, fibers of continuous glass filaments, and fibers of rock wool and slag wool are not classifiable with regard to their carcinogenicity to humans: Group 3.

The IARC working group chose not to make an overall evaluation of the newly developed fibers designed to be less biopersistent, such as the alkaline earth silicate or high-alumina, low-silica wools. This decision was based partly on the lack of human data (notwithstanding that the materials, in the cases where they have been studied, have been found to have low carcinogenic potential in experimental animals) and was partly due to the difficulties that the group encountered in trying to make a meaningful categorization of fibers based on their chemical composition.

It should be noted that, with regard to fibers of glass wool, rock wool and slag wool, the IARC has changed the assessments it made in 1988 (41).

Germany

In Germany, tests involving injection into the pleural or peritoneal cavities of laboratory animals are given greater weight than in most other European countries or the United States.

Appendix 3. Exposure data for refractory ceramic fibers.

Since refractory ceramic fibers are not produced in Sweden, there are no Swedish exposure data. There are, however, several studies from other countries. Esman *et al*. (24) studied exposures at three production facilities in the United States, and report factory averages of 0.05 to 2.6 f/ml. The highest measured value for a single task was 56 f/ml and was measured around post-production processing, including hand sawing and packaging in unventilated rooms. The large difference between factory averages was explained by differences in work methods and ventilation. In a European study by Cherrie *et al*. (14) covering 7 factories, the factory averages for processing workers ranged from 0.2 f/ml to about 7 times that: 1.36 f/ml. Several explanantions for the differences were proposed, including ventilation systems and manual handling procedures for the fiber material. The highest measured value, 3.4 f/ml, was reported in the group of 'secondary production'workers. Hori *et al*. (40) report averages of 0.27 – 0.66 f/ml around production of refractory ceramic fibers. These values were obtained by stationary monitors. Values obtained by personal monitors were reported to be at least twice as high.

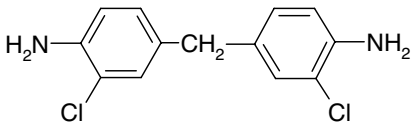
On the user side, Cheng *et al*. (11) have mapped fiber concentrations around both installation and removal of linings in high-temperature furnaces. Around lining removal the range of measured averages was 0.02 – 1.3 f/ml with a single peak of 17 f/ml. Around replacing the lining the range of average values was 0.14 – 0.62 f/ml, with a peak of 2.6 f/ml. Strübel (80) reports average fiber levels of 7.5 f/ml (1.06 – 23.0 f/ml) around furnace lining removal and 1.04 f/ml (0.38 – 2.45 f/ml) around replacing the lining. Krantz *et al*. (51) report a median content of 1.6 f/ml for a number of furnace lining removals in various smelters and foundries. A few extreme values of 210 f/ml were recorded around work under generally unventilated conditions. For replacing the furnace linings, the median value was 0.44 f/ml with a peak of 5 f/ml. The fiber concentrations for exposure category 1 (those directly exposed to the fibers) ranged from 0.26 – 1.2 f/ml (factory averages for the 4 studied factories).

With the exception of some extremely dusty post-processing tasks on the production side and removal of furnace linings on the use side, the reported exposure levels from both production and use are in the interval 0.1 – 2.6 f/ml, which should indicate that under normal circumstances dust conditions are probably comparable. On the other hand, the ACGIH (1) reports, with reference to 2 studies – Esman *et al*. (24) and Maxim *et al*. (63) – that 80% of the samples from production are below 1 f/ml and 90% of the samples from use are below 3 f/ml, which could indicate that, in general, use is dustier than production. Although any general conclusions should be drawn with caution, it can be said with some assurance that removal of used furnace linings and other dry manual work with refractory ceramic fibers can generate fiber concentrations of 10 f/ml or higher.

Consensus Report for 4,4´-methylene-bis(2-chloroaniline) (MOCA)

February 4, 2004

Chemical and physical data

CAS No:	101-14-4
Synonyms:	4,4´-methylene-di-(2-chloroaniline) 4,4´-methylene-bis-orthochloroaniline methylene-bis-ortho chloroaniline di(4-amino-3-chlorophenyl)methane 4,4'-diamino-3,3´-dichlorophenylmethane MBOCA
Formula:	$C_{13}H_{12}Cl_2N_2$
Structure:	
Molecular weight:	267.16 g/mol
Boiling point:	360 °C
Melting point	100 – 109 °C
Vapor pressure:	1 x 10^{-5} Pa at 25 °C, 0.0017 Pa at 60 °C
Saturation concentration:	0.0001 ppm at 25 °C
Solubility:	0.139 g/100 g water
Distribution coefficient:	log $P_{\text{octanol/water}}$ = 3.94

Uses

Pure methylene-bis(2-chloroaniline), or MOCA, at room temperature is a crystalline powder with a slight amine odor. It dissolves readily in alcohol, benzene and ether, but is much less soluble in water (17). MOCA is used primarily as a hardener in the manufacture of polymers and plastics, where it is added to modify the hardness, flexibility and impact resistance of the products (10).

Occupational exposure may occur via inhalation or skin uptake (30). The most important exposure pathway is probably through the skin, after contact with contaminated surfaces (6, 22). MOCA and its metabolites have been detected in

urine and plasma from workers exposed to MOCA (7, 38). Judging from the low MOCA levels measured in air samples from the work environments and the relatively high amounts of MOCA in urine samples from the workers, it is skin uptake rather than inhalation that is the primary path of absorption (22). Most occupational exposure occurs during production of MOCA or polymers containing MOCA (6, 22).

Uptake, biotransformation, excretion

Uptake

MOCA in workplace air occurs mainly as dust, and is absorbed via inhalation and through the skin. Several reports describe skin uptake as the primary path of exposure (6, 8, 22). Chin *et al*. showed that MOCA was rapidly taken up in neonatal foreskin *in vitro* during 4 hours. Uptake was most rapid during the first two hours and then declined. Uptake was dependent on temperature (5). In another study with human skin (from breast) uptake was small – 2.4 to 5.9% of the applied dose had been absorbed after 72 hours (16). There are no data from which to calculate quantitative skin uptake.

Attempts to estimate uptake of MOCA in work environments have been made, and air samples have shown relatively low levels of MOCA. The highest air concentrations were measured by personal samplers worn by workers in close contact with MOCA ($\leq 0.70\ \mu g/m^3$) and in floor dust near the melting vats ($1.9 \pm 2.9\ mg/m^2$). Air samples taken in the breathing zones of two mixers who handled MOCA directly (skin contact) were 0.34 and 0.06 $\mu g/m^3$. To estimate skin exposure, gauze pads were fastened to their hands. These bits of gauze were found to contain on average 24.6 ± 16 and 4.7 ± 2.4 μg MOCA after a workshift. These two workers also had relatively high levels of MOCA in urine (29.9 ± 15 and 94 ± 46 μg/l). Skin uptake was estimated in the same way for two other workers who mixed melted MOCA either by hand or automatically: MOCA concentrations in the gauze were 7.3 ± 9.5 and 3 ± 2.4 μg, and these workers had urine levels of around 15 μg MOCA/liter (6). The samples were taken over one workshift. In a similar study of a worker who (without protective clothing) mixed melted MOCA and was exposed to a MOCA mist, a 7- hour air sample showed a MOCA content of $8.9 \pm 0.5\ \mu g/m^3$. MOCA in urine of this worker ranged between 50 and 120 μg/g creatinine during the entire work week (18).

In a study with rats it was found that MOCA concentration was highest in liver 24 hours after a single oral dose of 281 μmol/kg b.w. MOCA concentrations were ranked as follows: liver > kidney > lung > spleen > bladder > testes > brain > lymphocytes (2).

Biotransformation

Biotransformation has been found to play an important role in the toxicity, genotoxicity and elimination of MOCA. Numerous MOCA metabolites have

been identified. MOCA is metabolized mostly in the liver by cytochrome P-450 enzymes (primarily CYP3A4) (1, 42). N-hydroxy MOCA has been identified as the most reactive and the primary carcinogenic metabolite (4). In addition to oxidation, the metabolism of MOCA involves conjugation with acetyl, sulfate and glucuronide groups (30). It has been suggested that MOCA conjugates formed in the liver are transported to the bladder where the acid environment hydrolyzes them to N-hydroxy MOCA (19). β-N-glucuronide MOCA has been identified as the primary metabolite in the urine of exposed workers (7). The MOCA-DNA adduct most commonly found in urine from MOCA-exposed workers is N-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol (19).

Excretion

MOCA is excreted in both urine and feces. The relative proportions reported in animal studies vary with species and method of administration. High levels of MOCA in the urine of workers have been reduced by introducing improved occupational hygiene, protective clothing, gloves, goggles and better ventilation. In addition, in many countries regular biological monitoring of MOCA in urine is used to detect any uptake (28). Workers with high amounts of MOCA in urine have usually been working in direct contact with MOCA, and often without using gloves (6, 30). Several analyses of urine from exposed workers have shown that the half time for MOCA in urine is about 23 hours (7, 28). The half time for MOCA-globin adducts in blood has been estimated to be 16 days, and the half time for MOCA-albumin adducts 4.6 days (2).

Rats (single i.p. dose of 13 or 100 mg/kg) and dogs (10 mg i.v.) given radioactively labeled MOCA excreted only a few percent of the dose in unmetabolized form (10, 23). A study with rats in which the labeled MOCA was given orally (5.5 – 5.6 mg/rat) showed that most MOCA is eliminated in the first 24 hours, primarily in feces (31 to 50%) and urine (16 to 27%) (26). In another study the substance (10 mg) was applied to the skin of dogs: of the total dose, 1.3% of the radioactivity was eliminated in urine and 0.62% in bile, and 90% remained in the skin after 24 hours (23). Only 0.4% of the MOCA detected in urine was unmetabolized. In another study, 2.5 mg radioactively labeled MOCA was applied to the skin of rats, and 2.5% of the radioactivity was excreted in urine within 72 hours. The rate of excretion was fairly constant for 3 days. MOCA apparently had a long retention time in the skin, however – 40 to 60% remained in the body after 72 hours (11).

Biological exposure monitoring

Gas chromatography/mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) are the most commonly used analysis methods for detecting MOCA in urine and as hemoglobin adducts in blood (30). Determination of MOCA in urine is best used for measuring exposure during a workshift. The amount of unmetabolized MOCA in urine is usually quite low. It is possible

to obtain higher levels of "free MOCA" by hydrolysis of urine samples containing MOCA. Analysis of free MOCA after hydrolysis is a recommended method for monitoring MOCA exposure using urine samples from workers (7). To estimate longer exposures, a method has been developed for quantitative analysis of MOCA bound to hemoglobin (3). There are no data correlating MOCA concentrations in air with those in urine.

Toxic effects

Human data

A few incidents of accidental exposure to MOCA have been reported. In 1987 a worker had MOCA spilled on his skin and involuntarily swallowed some of it. He later complained of eye irritation and nausea. Five hours after the exposure the MOCA level in urine was 1400 μg/g creatinine, and 23 hours after the exposure it had dropped to 30 μg/g creatinine. The urine sample taken 5 hours after the accident also contained large amounts of protein, possibly due to kidney damage (15).

In an accidental spill, a 30-year-old man had about 3 gallons (11 liters) of melted MOCA sprayed over his chest, back, arms and legs. The man was wearing protective clothing (with the sleeves rolled up) with face mask, protective gloves and goggles, and he showered for 45 minutes after the incident. He complained later of reddened, "sunburned" skin. A high level of MOCA (2169 μg/g creatinine) was detected in a urine sample taken 4 hours after the accident; 4 days later the level had dropped to 78 μg/g creatinine (28). The half time was calculated to be 23 hours (28). No effects other than the skin burn described above were reported.

Animal data

In cancer studies in which MOCA was given to rats in diet, weight gain was lower at both 500 and 1000 ppm. In these studies it was observed that at these dose levels life spans were significantly shorter for rats on both the standard diet and the low-protein diet (500 ppm was the highest concentration in the low-protein diet) (20). In a study with dogs, daily oral intake of 100 mg MOCA resulted in significantly higher levels of glutamic-pyruvic transaminase (GPT) activity, indicating liver damage, and increased numbers of erythrocytes, leukocytes and epithelial cells in urine (36). Another study reports elevated mortality in female mice receiving high doses of MOCA in diet (2000 ppm). There was also reduced weight gain in rats receiving 1000 ppm in diet (31), see also Table 1.

Mutagenicity, genotoxicity

MOCA has been shown to be genotoxic and mutagenic in both *in vitro* and *in vivo* tests. MOCA was mutagenic in *Salmonella typhimurium* strains TA98 and TA100

only after activation with S-9 mixture from hepatic cells (13, 21, 29). The N-acetyl metabolites were not mutagenic under the same conditions (13). MOCA has been found to induce DNA repair in hepatocytes from rats, mice and hamsters (24, 25). In addition, lymphocytes from workers exposed to MOCA (supervisors, laboratory personnel and production workers) had higher numbers of sister chromatid exchanges than those from unexposed controls. Precise exposure data are not given. Elevated numbers of sister chromatid exchanges were also observed in lymphocytes from rats exposed to MOCA (5 daily i.p. injections of 125 or 250 mg/kg b.w.) (9).

Examination of exfoliated urothelial cells in the urine of MOCA-exposed workers revealed that they contained significantly higher numbers of micronuclei than those from controls. The MOCA workers had various jobs at the factory, and most of them worked with polyurethane casting. MOCA concentrations in urine from the workers ranged from 0.4 to 48.6 μmol/mol creatinine, whereas urine from controls contained no MOCA at all. There was no correlation between the micronuclei and MOCA levels in urine. In this study, smoking had no discernible effect on the number of micronuclei in either in the exposed workers or in the controls. There were only a few smokers in the study, however (5 in each group) (27).

MOCA-DNA adducts and MOCA-hemoglobin adducts have been identified in several studies (3, 23, 33, 34). Urine from a worker involuntarily exposed to MOCA was analyzed for MOCA-DNA adducts. High levels of adducts were observed in the urine 4 hours after the exposure. A dramatic drop in DNA adducts in the urine was observed during the following 17 hours, after which the rate of reduction slowed (19).

Carcinogenicity

The International Agency for Research on Cancer (IARC) has classified MOCA as "probably carcinogenic to humans" (Group 2A) (17). The evidence from animal experiments is regarded as sufficient to justify this classification despite the lack of evidence that MOCA causes cancer in humans. The results of cancer studies with rats, mice and dogs are summarized in Table 2 and below.

Human data

Three cases of bladder cancer in MOCA workers have been reported. Urine and cell samples from 385 (of a total of 552) workers at a MOCA production plant were analyzed. The median interval between initial exposure and the time the samples were taken was estimated to be about 11.5 years. Sixteen of the workers had blood in urine and 21 had atypical cell samples. After the first cancer case was discovered, 200 workers were given a more thorough examination with cystoscopy. Two more cancer cases were discovered, although neither of these workers had blood in urine (hematuria). All three subjects had been employed at the plant for only a short time (1.5 months to 1 year) but had probably been

exposed to high levels of MOCA. Two of the workers, 28 and 29 years old, were non-smokers, and according to the report had never worked with other chemicals suspected of causing bladder cancer. The latency times from initial exposure to diagnosis were 8 and 11 years. The third worker was 44 years old, a smoker, and had previously worked with other chemicals. The latency time between initial exposure and diagnosis was 16 years (39, 40). At the request of the Polyurethane Manufacturers Association (PMA), Hogan (14) tried to reconstruct exposures in these three cases. He arrived at the conclusion that the workers may have been exposed to other carcinogens, including other aromatic amines. Hogan's exposure estimates have been questioned (41).

Animal data

MOCA has been shown to cause cancer in rats that are given the substance in diet. A low-protein diet yielded a higher tumor incidence than the standard diet, and it has therefore been suggested that protein plays a protective role in MOCA-induced cancer. The tumors occurred primarily in lungs, liver and breast (35), see also Table 2. Russfield *et al*. demonstrated that MOCA induced liver tumors and hemangiosarcomas in mice, and lung and liver tumors in rats (31). Dogs developed bladder cancer after 9 years of exposure to MOCA (36). Grundmann *et al*. showed that MOCA in the diet induced lung and liver tumors in rats (12), see also Table 2**.**

In vitro studies have shown that N-hydroxy MOCA can transform non-tumorigenic cell lines to tumorigenic ones. Injection of these cells into naked mice induced tumors within 17 weeks (37).

Effects on reproduction

No teratogenic or other effects on reproduction have been reported.

Dose-effect / dose-response relationships

There are no data on which to base a dose-effect or dose-response relationship for either human or animal exposures. Toxic and carcinogenic effects observed in animal experiments are summarized in Tables 1 and 2.

Conclusions

There are not sufficient data for defining a critical effect for MOCA. Judging from animal experiments, the critical effect is cancer. MOCA exposure is primarily via skin uptake. MOCA is genotoxic *in vitro* and forms DNA adducts *in vivo*. MOCA is carcinogenic to experimental animals and should be regarded as carcinogenic to humans.

Table 1. Toxic effects of experimental exposure to MOCA.

Species	Dose, exposure method	Exposure time	Effects	Ref
Mouse HaM/ICR 25 males 25 females	0, 1000, 2000 ppm in standard diet per os	18 months	2000 ppm females: elevated mortality	31
Rat ChR-CD 25 males	0, 500, 1000 ppm in standard diet per os	18 months	1000 ppm: lower weight gain	31
Rat ChR-SD 100, 100, 75, 50 males	0, 250, 500, 1000 ppm in standard diet per os	18 months	500 ppm: lower weight gain, shorter life span* 1000 ppm: lower weight gain, shorter life span*	20
Rat ChR-SD 100, 100, 75, 50 males	0, 125, 250, 500 ppm in low-protein diet per os	18 months	500 ppm: shorter life span*	20
Beagle 6 females**	0, 100 mg/day in capsule per os	9 years	100 mg/day: elevated GPT activity*, elevated erythrocytes, leukocytes and epithelial cells in urine	36

* significantly different from controls
** one of which died early

Table 2. Results of cancer tests with laboratory animals.

Species	Dose, exposure method	Exposure time	Effects	Ref
Mouse HaM/ICR males females	0, 1000, 2000 ppm in standard diet per os	18 months	0 ppm males: hepatoma 3/18, pulmonary adenoma 5/18 1000 ppm males: hepatoma 3/13, hemangioma 2/13, hemangiosarcoma 1/13, pulmonary adenoma 3/13 2000 ppm males: hepatoma 4/20, hemangioma 5/20, hemangiosarcoma 3/20, pulmonary adenoma 2/20, pulmonary adenocarcinoma 2/20 0 ppm females: hemangioma 1/20, pulmonary adenoma 4/20, pulmonary adenocarcinoma 2/20, lymphosarcoma 4/20, reticulosarcoma 6/20 1000 ppm females: hepatoma 9/21*, pulmonary adenoma 3/21, lymphosarcoma 3/21, reticulosarcoma 3/21 2000 ppm females: hepatoma 7/14*, hemangioma 4/14, hemangiosarcoma 2/14, pulmonary adenoma 2/14, lymphosarcoma 1/14	31
Rat Wistar males females	1000 ppm in low-protein diet, total dose 27 g/kg per os	535-565 days	0 ppm males, females: breast fibroadenoma 2/50 1000 ppm males: multiple hepatoma 22/25, primary lung tumor 8/25 1000 ppm females: multiple hepatoma 18/25, primary lung tumor 5/25	12
Rat ChR-CD males	0, 500, 1000 ppm in standard diet per os	18 months	0 ppm: cholangiocarcinoma 1/22, pulmonary adenoma 1/22 500 ppm: hepatoma 1/22, pulmonary adenomatosis 3/22, pulmonary adenoma 1/22, pulmonary adenocarcinoma 1/22, bladder cancer 2/22, glioma 1/22 1000 ppm: hepatoma 4/19, pulmonary adenomatosis 4/19, pulmonary adenoma 1/19, pulmonary adenocarcinoma 1/19, stomach adenocarcinoma 1/19, sebaceous adenoma in ear 1/19	31
Rat ChR-CD males females	0, 1000 ppm in standard diet per os	548-560 days	0 ppm males: pulmonary adenomatosis 1/44, leukemia 2/44, lymphoma 2/44, breast fibroadenoma 1/44, pituitary adenoma 4/44. 1000 ppm males: pulmonary adenomatosis 14/44*, pulmonary adenocarcinoma 21/44*, pulmonary squamous-cell carcinoma 1/44, pleural tumor 4/44, pleural lipoma 1/44, hepatocellular adenoma 3/44, hepatocellular carcinoma 3/44, leukemia 1/44, lymphoma 4/44, breast fibroadenoma 1/44, breast adenocarcinoma 3/44, pituitary adenoma 4/44, interstitial adenoma in testes 4/44, renal adenoma 1/44 0 ppm females: pulmonary adenomatosis 1/44, pleural lipoma 1/44, lymphoma 1/44, breast fibroadenoma 17/44, breast adenocarcinoma 3/44, breast fibrosarcoma 1/44, pituitary adenoma 12/44 1000 ppm females: pulmonary adenomatosis 11/44*, pulmonary adenocarcinoma 27/44*, pulmonary squamous-cell carcinoma 1/44, pleural tumor 2/44, hemangioma 1/44, hepatocellular adenoma 2/44, hepatocellular carcinoma 3/44, cholangioma 1/44, leukemia 1/44, lymphoma 3/44, breast fibroadenoma 18/44, breast adenocarcinoma 5/44, pituitary adenoma 1/44*, vaginal fibrosarcoma 1/44, renal adenoma 1/44, renal adenocarcinoma 1/44	35

Table 2. Continued.

Species	Dose, exposure method	Exposure time	Effects	Ref
Rat ChR-CD males females	0, 1000 ppm in low-protein diet per os	400 - 423 days	0 ppm males: pulmonary adenomatosis 1/21 1000 ppm males: pulmonary adenomatosis 8/21*, pulmonary adenocarcinoma 5/21*, pleural tumor 1/21, hepatocellular adenoma 5/21*, hepatocellular carcinoma 11/21*, skin squamous cell carcinoma 1/21, lymphoma 1/21, renal lipoma 1/21, ganglioneuroma 1/21 0 ppm females: pulmonary adenomatosis 1/21, breast fibroadenoma 7/21 1000 ppm females: pulmonary adenomatosis 14/21*, pulmonary adenocarcinoma 6/21*, pleural tumor 1/21, hepatocellular adenoma 2/21, hepatocellular carcinoma 1/21, breast fibroadenoma 1/21*, breast adenocarcinoma 6/21*, peritoneal lipoma 1/21, ileal adenocarcinoma 1/21	35
Rat ChR-SD males	0, 250, 500, 1000 ppm in standard diet per os	18 months	0 ppm: primary lung tumors 1/100, breast adenocarcinoma 1/100, Zymbal's gland tumor 1/100, hemangiosarcoma 2/100, pituitary adenoma 42/100 250 ppm: pulmonary adenocarcinoma 14/100*, primary lung tumor 23/100*, breast adenocarcinoma 5/100, Zymbal's gland tumor 8/100*, hepatocellular carcinoma 3/100, hemangiosarcoma 4/100, pituitary adenoma 36/100. 500 ppm: pulmonary adenocarcinoma 20/75*, primary lung tumor 28/75*, breast adenocarcinoma 8/75*, Zymbal's gland tumor 5/75, hepatocellular carcinoma 3/75, hemangiosarcoma 3/75, pituitary adenoma 19/75* 1000 ppm: pulmonary adenocarcinoma 31/50*, primary lung tumor 35/50*, breast adenocarcinoma 14/50*, Zymbal's gland tumor 11/50*, hepatocellular carcinoma 18/50*, hemangiosarcoma 0/50, pituitary adenoma 2/50*	20
Rat ChR-SD males	0, 125, 250, 500 ppm in low-protein diet per os	18 months	0 ppm: hemangiosarcoma 1/100, pituitary adenoma 23/100 125 ppm: pulmonary adenocarcinoma 3/100, primary lung tumor 6/100*, breast adenocarcinoma 1/100, Zymbal's gland tumor 0, hepatocellular carcinoma 0, hemangiosarcoma 2/100, pituitary adenoma 16/100 250 ppm: pulmonary adenocarcinoma 7/75*, primary lung tumor 11/75*, breast adenocarcinoma 3/75, Zymbal's gland tumor 4/75*, hepatocellular carcinoma 0, hemangiosarcoma 4/75, pituitary adenoma 9/75* 500 ppm: pulmonary adenocarcinoma 8/50*, primary lung tumor 13/50*, breast adenocarcinoma 3/50*, Zymbal's gland tumor 6/50*, hepatocellular carcinoma 9/50*, hemangiosarcoma 4/50*, pituitary adenoma 10/50	20
Beagles females	0, 100 mg/day in capsules per os	9 years	Controls: Breast nodules 5/6, breast adenocarcinoma, carcinoma or carcinosarcoma 4/6 100 mg/day: bladder cancer (papillary transitional cell carcinoma) 4/5*, urethra (transitional cell carcinoma and adenocarcinoma) 1/5, liver nodules 3/5	36

* significantly different from controls.

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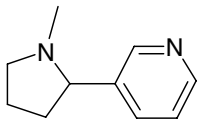
Consensus Report for Nicotine

June 2, 2004

This document is based on published articles registered in the data bases Medline and Toxline through 2003 and on recently published reviews of the literature.

Chemical and physical data. Use

CAS No.:	54-11-5
Synonym:	3-(1-methyl-2-pyrrolidinyl)pyridine
Formula:	$C_{10}H_{14}N_2$
Structure:	
Molecular weight:	162.2
Density:	1.01 (20 °C)
Boiling point:	247 °C
Melting point:	- 79 °C
Vapor pressure:	5.7 Pa (20 °C)
Saturation concentration:	56 ppm
Distribution coefficient: ($\log P_{octanol/water}$)	1.17
Conversion factors:	1 mg/m^3 = 0.149 ppm (20 °C) 1 ppm = 6.73 mg/m^3 (20 °C)

Nicotine is a tertiary amine with a pyridine and a pyrrolidine ring. It is an oily, colorless/pale yellow to dark brown liquid, and has a fishy odor when heated. It is hygroscopic and forms water-soluble salts. Nicotine is a weak base, with a pKa of 7.9.

Background exposure

In assessing nicotine exposure in work environments, it is essential to know the background exposure. Nicotine is a drug used regularly – usually daily – by a third to a half of the world's population. This portion of the population has extremely high nicotine exposure from smoking cigarettes, cigars and pipes, taking snuff and chewing tobacco. A single cigarette yields a dose of about 1 to 1.2 mg nicotine. Most of the population is exposed to nicotine daily via "passive smoking": exposure to environmental tobacco smoke (ETS). On average,

however, this exposure is only 1/100 to 1/1000 of the exposure of a smoker. Exposure to environmental tobacco smoke can be particularly high if people smoke in small, closed-in spaces such as automobiles or homes. If a home contains two smokers, the total nicotine dose from ETS is on average about 10 times that in a home in which there are no smokers. There are numerous studies and exposure data on passive smoking in home environments (92). Background exposure to nicotine in indoor air is about 3 μg/m^3 (93), but can be much higher in indoor environments containing smokers (95, 97). In one study it was found that the air content of nicotine in a bus where smoking had previously occurred was about 30 μg/m^3 due to the dust from the seats and other interior surfaces (93). Nicotine content in food, on the other hand, does not make a measurable contribution to the nicotine or cotinine in body fluids (94). A median value for cotinine content in the urine of people who neither smoke nor use snuff, do not work in a smoky environment and are not exposed to passive smoking at home is 2 μg/l (92), see also Table 1. A group that completely avoided exposure to environmental tobacco smoke (in an experimental situation) had a level one tenth of that (93).

Table 1. Biological exposure measurements: cotinine in plasma and urine after nicotine exposure from cigarette smoking, environmental tobacco smoke, tobacco harvesting and production of pharmaceuticals containing nicotine.

Exposure	Plasma cotinine (μg/l)	Urine cotinine (μg/l)	Ref.
Environmental tobacco smoke 110 μg/m^3, 2 hours		13	93
Environmental tobacco smoke 110 μg/m^3*		180	92
Environmental tobacco smoke 500 μg/m^3*		730	92
Environmental tobacco smoke 10 μg/m^3* (restaurant work 8 hrs/day)		16	91, 92
Smoking 3.9 cigarettes per day (1 cig. ≈ 1 – 1.2 mg absorbed dose)	54		14
Smoking 5 cigarettes per day	70		14
"Smokers" (plasma nicotine = 20 – 30 μg/l)	>300	>2,500	14, 90
Production of nicotine pharmaceuticals (skin absorption + inhalation) (plasma nicotine = 0 – 0.3 μg/l)		3 – 70	96
Tobacco harvesters (symptom-free)	15	100	24

*at steady state, after about 100 hours

Nicotine exposure in work environments

Working with tobacco, especially tobacco harvesting (skin uptake) and spraying nicotine as an insecticide (uptake from skin and digestive system), can result in high nicotine exposure, but in Sweden such jobs are virtually non-existent. There are a few published reports with data on nicotine uptake due to exposure to tobacco leaves (see Table 1). The nicotine content in dew from tobacco leaves was reported in one study to be 1.9 μg/l; the tobacco leaves themselves contained 20 mg/g (24). Tobacco harvesters are known to have high nicotine exposure (39, 60). Measurements of cotinine in saliva indicated that the harvesters had higher nicotine exposure than other tobacco workers (72).

However, the pharmaceutical industry has now begun to process nicotine (skin uptake, inhalation). There are no published exposure data, but some information can be found in internal company reports; there is also a study from manufacture of pharmaceutical products to help people quit smoking (96), see Table 1. In production of nicotine patches, the highest exposure is associated with powder mixing (the nicotine is bound to an ion exchanger, nicotine resinate). The material is mixed inside closed boxes where the measured air concentration was 20,000 $\mu g/m^3$. The exposure of workers outside the boxes is 100 – 200 $\mu g/m^3$ (96). The concentration in the breathing zone around receiving the finished patches is at most 100 $\mu g/m^3$. To avoid the heavy skin exposure associated with packaging the nicotine patches, workers wear double nitrile gloves and change them every 20 minutes (101).

There are also new data on nicotine exposure (inhalation) via environmental tobacco smoke. Restaurant workers in particular are likely to be exposed to high levels of ETS, since they often work in crowded, poorly ventilated rooms where passive smoking can be high – although it usually does not exceed 100 $\mu g/m^3$ air. There are some exposure data on passive smoking in work environments (see Table 1). In general, nicotine exposure during work in smoky environments is 10 to 100 times higher than in work where no smoking occurs (28, 58, 91, 92).

It is clear from the above that occupational exposure to nicotine varies over a wide range.

Uptake, biotransformation, excretion

Uptake of nicotine through the skin depends on the nicotine concentration, the pH and the solvent. For example, an application of 50% nicotine in water is absorbed about 15 times faster than pure nicotine, and a 1% solution buffered to pH 7 yields the same uptake as pure nicotine. Uptake is reduced if the pH is lowered or if the nicotine is dissolved in ethanol (101). One study (101) reports that human skin *in vitro* absorbs pure nicotine at the rate of 82 $\mu g/cm^2$/hour. Applying ECETOC's criteria for skin notation (29), i.e. exposure of 2000 cm^2 skin (equivalent to hands and lower arms) for 1 hour (equivalent to exposure of 250 cm^2 skin, or 70% of a

hand, for 8 hours) and using the absorption rate above yields a dermal absorbed dose of 164 mg. This is about 70 times as much as the 2.5 mg resulting from 8 hours of exposure to the SCOEL (Scientific Committee for Occupational Exposure Limits) limit of 0.5 mg/m^3 (32) (assuming inhalation of 10 m^3 air in 8 hours and 50% absorption in the lungs). Skin exposure to nicotine can thus result in significant absorption with systemic effects.

Nicotine is absorbed very rapidly from the lungs, and with cigarette smoking the blood concentration peaks after only 10 to 20 seconds (12). Uptake via mucous membranes in the mouth (chewing tobacco, snuff, nicotine chewing gum) is much slower, and blood concentration rises slowly to a maximum value after about 30 minutes. Data on uptake via the human digestive tract are much less abundant, but nicotine is absorbed readily (1).

After absorption in the lungs, nicotine concentrates in the brain, kidneys, stomach, adrenal medulla, nose and salivary glands (13).

Nicotine passes the placental barrier and has been measured in amniotic fluid. It also passes into breast milk (13). Nicotine concentrations in amniotic fluid, placenta and plasma from umbilical blood have been found to exceed the plasma nicotine level in the mother (59).

Nicotine is metabolized rapidly and extensively, primarily in the liver but also to some extent in lungs and kidneys. Excretion via kidneys depends on pH and urine flow, and in humans accounts for 2 to 35% of total elimination (11). The half time of nicotine in blood, urine and saliva is about 2 hours (36). The primary metabolites are cotinine and nicotine-N-oxide. Cotinine has a half time of about 19 hours (93).

Biological measures of exposure

Using air measurements to monitor nicotine exposure can be misleading, since uptake is often predominantly via the skin. Cotinine has been found to be an excellent biomarker for nicotine exposure (93). Excellent methods for determining cotinine, notably gas chromatography/mass spectrometry, have been in use for several years, and mass spectrometry yields accurate results below the μg/liter level (77). Methods have been published for calculating the nicotine dose using cotinine in urine (U-cotinine) (43, 92), plasma (24) and blood (14):

$$\text{daily nicotine dose (mg)} = [\text{plasma cotinine}_{ss}\ (\mu g/l)] \times 0.08 \quad (24)$$

Since steady state (ss) levels, which are attained after about 100 hours, are proportional, daily nicotine intake can thus be calculated from the cotinine content in plasma, saliva or urine. The daily nicotine dose can be calculated in mg by multiplying plasma cotinine (in μg/l) by 0.08 (see above equation) (24), or multiplying the cotinine content in urine (in μg/l) by 0.012 (92, 94). Correcting for creatinine or density is not important, and can even be misleading at the individual level. It has little effect on the average level of cotinine in urine (μg/g creatinine is

about the same as μg/l). It is best to standardize sampling time, using morning urine samples. In one study the saliva:plasma ratio was 1.1 (94).

Manufacture of nicotine-containing pharmaceutical products designed to help people quit smoking was associated with urine cotinine levels ranging from 3 to 70 μg/l, depending on the task. The highest level corresponds to a nicotine dose of 0.8 mg/day from skin and inhalation exposure (see Table 1) (96). Since the workers in this study wore protective clothing and double nitrile gloves, it is likely that most of this uptake was from inhalation.

Ten healthy tobacco workers (and 5 controls) wearing good protective clothing (raincoats and rubber gloves) had greatly elevated nicotine levels in blood after a workshift (average 3.5 μg/l). Cotinine levels were 15 μg/l in plasma and 100 μg/l in urine. Calculating with the formula given above, this corresponds to a daily dose of 1.2 mg (24), or smoking 1 cigarette per day. Working conditions were described as good. Cotinine levels about 10 times higher (range 30 – 3000 μg/l) are reported by Gehlbach *et al*. (39) in tobacco harvesters, 25% of whom had symptoms. Even higher levels, and a higher prevalence of symptoms (53%) are reported in a study by Ghosh *et al*. (40). This study, however, is marred by poor control of smoking habits and protective clothing, and by analysis method.

The median value for urine cotinine in a group of restaurant employees was about 16 μg/l (range 10 – 28 μg/l) (91). This value would indicate an average nicotine exposure of 10 $\mu g/m^3$ during an 8-hour workday (urine cotinine $_{\text{maximum value}}$ = 30 μg/l) with a 16-hour exposure-free interval (calculated backward from results of experimental exposure to environmental tobacco smoke) (92).

For assessing nicotine exposure, cotinine in urine provides a more reliable estimate of actual dose than air concentration because of the risk of substantial skin uptake.

Toxic effects

Acute poisoning

The high toxicity of nicotine has been known for a century. There are many reported cases of acute poisoning, most of them due to oral or skin uptake – especially in the 1920s and 1930s when nicotine (nicotine sulfate) was a popular insecticide. Some fatal occupational exposures have been reported. Symptoms of severe poisoning can appear within a few minutes. The LD_{50} for oral intake of nicotine is reported to be 3.3 mg/kg for mice and 53 mg/kg for rats (56). Although there is no precise documentation, the lethal dose for humans is taken to be around 50 to 60 mg (56). There are several reported cases of poisoning due to suicide attempts with nicotine patches (99). Severe poisoning causes convulsions, heart arrhythmia and death within a few minutes to an hour.

With less severe poisoning, the usual symptoms are nausea, vomiting, diarrhea, headache, confusion, rapid heartbeat, elevated blood pressure and excessive salivation. Slight to moderate nicotine poisoning is not uncommon in processing

workers and insecticide users. The most common form, Green Tobacco Sickness (GTS), has been described in several studies of tobacco field workers (39, 40, 60, 71). The symptoms are dizziness, vomiting, headache, weakness, stomach pains, cramps, salivation and sweating, and frequently occur among non-smokers – often in up to half of a group of tobacco workers. Uptake occurs via the skin. The workers who picked tobacco leaves had the highest cotinine levels (average 890 μg/g creatinine, which is about the same in μg/l), and 25% of these had GTS symptoms (39). Workers who wore plastic raincoats had much lower values. The workers with symptoms (dizziness and nausea) were found to have elevated excretion of nicotine and cotinine (39, 40), but the level associated with the appearance of symptoms was not given. People who are unaccustomed to smoking and smoke 3 to 6 cigarettes within an hour get the same symptoms, and their nicotine/cotinine levels are about the same as those of tobacco harvesters with symptoms. Symptoms (runny eyes and blurry vision) were reported in significantly higher frequency ($p < 0.04$) in non-smoking tobacco growers with urine cotinine levels of ≥ 50 μg/l/m^2 (range 50 – 290 μg/l/m^2) when they were compared with workers who had levels below 50 μg/l/m^2. The correlation was seen only after the urine cotinine concentration had been corrected with body surface area (68). The authors propose that the symptoms are due to GTS, but observe that they may also be due to exposure to organic phosphates.

Acute and chronic cardiovascular effects

There is a lot of information, both human data and results from experiments with laboratory animals, about exposure and cardiovascular effects of cigarette smoking (53). The rapid nicotine uptake and high levels that result from smoking are believed to stimulate the sympathetic nervous system and produce systemic catecholamine secretion with effects on hemodynamics (17). Although there are little human data on the cardiovascular effects of exposure to nicotine alone, there are some animal data.

As a rule, high doses, similar to those from smoking, have been used in animal studies. Only a few give measured nicotine levels in plasma, but levels around 25 μg/l (the levels seen in smokers) are reported at doses ranging anywhere from 0.5 to 2 mg/kg/day (10). Male rats were given nicotine in drinking water in 3 groups: 0 (n = 80), 1.14 (n = 100) and 4.56 (n = 100) mg/kg/day for 34 weeks and then exposed to hypoxia (89). With hypoxia treatment, there was an elevation in mortality at the highest nicotine dose, or about the level measured in heavy smokers (>20 cigarettes/day). No effect on hematocrit was seen. In other animal studies, high intramuscular (i.m.) or subcutaneous (s.c.) injections of nicotine have resulted in changes in plasma lipoproteins (37, 84). Similar changes in lipoproteins have been observed in smokers (23, 38). Only minimal changes were seen in human studies with transdermal nicotine applications, however (4, 73). Rabbits with hypercholesterolemia that were given intramuscular or subcutaneous injections of high doses (11 mg/kg/day) developed atherosclerosis (84). Their cholesterol levels, however, were higher than those regarded as clinically relevant.

Lower doses of nicotine (1 mg/kg/day) and a low-cholesterol diet had no effect in a study by Fisher *et al*. (37). The vascular toxicity of nicotine has been clearly shown in animal experiments with exposures higher than those associated with smoking. The effect on smooth muscle morphology in cultured arterial smooth muscle cells was dose-dependent and quite small at levels associated with smoking (85). In an inhalation experiment, rats (n = 68) were exposed to 0.5 mg/m^3 nicotine 20 hours/day, 5 days/week for two years and compared with controls (n = 34). Mortality and arteriosclerosis were no higher among the exposed rats, but they weighed less than controls. The exposure resulted in plasma cotinine levels above 100 μg/l (about double that of a smoker) (88).

Ahmed *et al*. (2) report abnormal left ventricle function and interstitial fibrosis in the hearts of beagles (18 months old at the start of the study) after 22 months of exposure to cigarette smoke via tracheostoma (7 cigarettes/day, equivalent to 377 μg/kg; n = 9) or to nicotine alone via intramuscular injection (210 μg/kg, twice a day; n = 8), when compared with controls (n = 7). The fact that the effects were observed in both treated groups suggests that nicotine has a role in cardiovascular effects.

There are only a few human studies. In a large prospective study of construction workers, an elevated mortality due to heart and circulatory diseases was identified for snuff users (19). The risk, however, was lower than that for smokers. The relative risk (RR) was 1.4 (95% CI 1.2 – 1.6) for snuff users (compared with a group that did not use tobacco) and 1.9 (95% CI 1.7 – 2.2) for smokers (> 15 cigarettes/day); see Table 2. The analysis included only snuff users who had never smoked. Mixed tobacco use or pipe smoking was not studied. In a smaller retrospective case-control study of patients with myocardial infarctions, however, Huhtasaari *et al*. (47) found no elevated risk for snuff users. In this study the relative risk for cigarette smokers was 1.87 (95% CI 1.40 – 2.48) and for snuff users 0.89 (95% CI 0.62 – 1.29). 'Tobacco users' were defined as anyone smoking or taking snuff at least once a day. This study, like the others, includes no analysis of mixed tobacco use or other nicotine use. Another study by Huhtasaari *et al*. was also negative regarding a correlation between snuff use and cardiovascular effects (48).

Hansson *et al*. (42) had healthy subjects inhale aerosols of nicotine solutions (1 – 8 mg/ml; 0.01 ml/inhalation). In one study each subject inhaled 21 times in 5 minutes, inhaling a total of 0, 0.4, 0.8 or 1.7 mg nicotine. Effects on systolic blood pressure and heart rate were observed during a subsequent 30-minute observation period even after the lowest dose (0.4 mg). Coughing was also observed after a single inhalation of a solution containing 4 mg nicotine/ml. The lowest dose that triggered coughing was 0.04 mg. The authors conclude that nicotine stimulates the coughing reflex locally via nerve ends in the bronchial mucosa (see also Respiratory effects and hypersensitivity).

Snuff users have about the same average nicotine exposure as smokers (90). It can be assumed that the harmful effects of nicotine are greater with the lung exposure from smoking than with other methods/pathways of administration,

since high arterial peak concentrations are reached more quickly (16). Further, it is generally accepted that the effects of smoking are due to the combined action of nicotine and several other substances in the smoke, such as carbon monoxide (16). It is thus not clear how large a role nicotine plays in cardiovascular disease. It has been shown that nicotine, at levels about the same as those found in smokers and snuff users (plasma nicotine > 10 – 20 μg/l), stimulates the sympathetic nervous system, accelerates heart rate and raises blood pressure, and can also affect lipid metabolism and cause endothelial damage related to development of arteriosclerosis. There is no convincing evidence of nicotine effects at lower exposure (17).

The central nervous system and nicotine addiction

The effects of nicotine on the CNS are complex (15), see also Effects on reproduction. Alertness and relaxation are affected. Because of the dependency-inducing nature of nicotine most smokers can not quit, although in several studies the majority of them have expressed a desire to do so. Nicotine changes the spectrum of EEG activity (69). Activation of nicotinic acetylcholine receptors (nAChRs) in the CNS is related to dependence (15). Studies with rats have shown that the mesolimbic dopamine system is central to the dependency-inducing effect of nicotine. Dose, rate of uptake and tolerance development are the most important factors in nicotine addiction. The rapid uptake with inhalation is probably correlated to the addictive effect more closely than is the amount of nicotine (13). Inhalation provides the quickest and highest exposure in the brain. Smoking one cigarette for about 5 minutes (equivalent to an absorbed dose of about 1 – 1.2 mg nicotine) gives an initial peak concentration in the brain within 10 to 20 seconds after the first puff. It can be expected that there is a less addictive effect of 8 hours of occupational exposure to the same dose a smoker gets within 5 minutes. Support for this is that nicotine patches are less dependency-producing than other nicotine preparations (70). The threshold for the dependency-producing effect is poorly documented. Benowitz (12) states that there is a risk of dependency with smoking 2 cigarettes per day. On the other hand, the same author states in a later publication that the threshold dose for inducing dependency is probably about 5 mg nicotine per day (though this statement is unsupported). The author refers to a group of smokers (about 10%) who smoke fewer than 5 cigarettes per day and don't seem to be dependent (14). These individuals can go without smoking for one or several days without abstinence symptoms. Five mg nicotine per day should, according to the formula given earlier (under Biological measures of exposure), yield cotinine levels of about 60 μg/l plasma and about 400 μg/l urine.

Other effects

A large number of other effects have been reported at high exposure levels:

Respiratory effects and hypersensitivity

Inhaled nicotine causes a dose-dependent coughing and airway constriction similar to the effects of capsaicin. High exposures cause severe coughing and breathing difficulty (42), see also Table 2. In pharmaceutical production, cleaning the work spaces/machines where powder containing nicotine (nicotine resinate or nicotine ß-cyclodextrin) is processed often leads to coughing attacks (96). Nicotine in very high doses is strongly irritating to skin and eyes: it can cause severe eye irritation in pharmaceutical production workers dealing with a machine malfunction in a closed system (see Nicotine exposure in work environments) if they do not wear protective goggles. There are several case reports of local vasculitis caused by nicotine patches (87).

Neuromuscular effects

Nicotine stimulates Renshaw cells, reducing muscle tonus in spastic patients. On the other hand, nicotine can also increase EMG (electromyogram) activity in the trapezius muscle (33).

Endocrine effects

The levels of the catecholamines adrenaline and noradrenaline in plasma rise after cigarette smoking (17). Acetylcholine, vasopressin, growth hormone, dopamine, serotonin, cortisol and ACTH levels also rise with smoking, which is assumed to be related to nicotine (9, 12).

Mutagenicity, carcinogenicity

There is an enormous amount of documentation on the risks of smoking; among other things, there are a large number of carcinogens in tobacco smoke (45, 49). Until recently, it was assumed that nicotine alone was not carcinogenic and was of no importance in the etiology of tobacco-related cancer (12). ETS exposure was also connected to an elevated risk of lung cancer (50), but it was not associated with nicotine. The past few years, however, have provided evidence that nicotine can play an important role in carcinogenesis by acting as a growth promoter, inhibiting apoptosis and inducing oxidative stress leading to DNA damage (see References 8 and 21 and references cited therein).

No increase in tumor frequency was observed in a 2-year inhalation study in which rats were exposed to twice the amount of nicotine absorbed by a smoker (88). The nicotine exposure was 0.5 mg/m^3, 20 hours/day, 5 days/week. The plasma nicotine level in the animals was a bit over 100 µg/l, more than twice that of a smoker.

Nicotine has been negative in most short-term tests for mutagenicity and genotoxicity. Induction of sister chromatid exchanges (SCE) in CHO cells was not seen in a study made by Doolittle *et al*. (27), but was observed in a study by

Trivedi *et al*. (86). Nicotine was negative in Ames' tests with *Salmonella typhimurium* in several studies (20, 26, 27, 61). Nicotine had no effect in a bacterial genotoxicity test based on chemiluminescence, but the test was positive for cotinine (100).

A large prospective study of construction workers (mentioned above) revealed no increase in cancer risk for the subjects who used snuff (19) (see also Table 2). A Swedish case-control study on the connection between snuff and cancers of the mouth, throat and esophagus revealed that snuff use had little or no effect on the occurrence of these forms of cancer. If only persons who had never smoked were included, there were only 9 cases and 10 referents who used snuff alone. The relative risk was 10.5 (95% CI 1.4 – 117) for former snuff users and 3.3 (95% CI 0.8 – 12.0) for current snuff users (57). An earlier study from northern Sweden had shown a tendency to elevated risk of lip cancer in former snuff users (RR = 1.8, 95% CI 0.9 – 3.7) (75).

Effects on reproduction

No studies were found dealing with toxic effects on human reproduction caused by exposure to nicotine alone. In Sweden, nicotine as a pharmaceutical is placed in category C with regard to pregnancy (substances which are known to, or on good grounds are presumed to, pose a risk to the fetus or newborn, without being directly teratogenic) and in group III with regard to nursing (substances which, at therapeutic doses, enter milk in amounts large enough to pose a risk of effects on the child) (34).

Exposure to tobacco smoke has been associated with several toxic effects on human reproduction: placenta previa, premature detachment of placenta, premature birth, intrauterine growth restriction, low birth weight, multiple malformations, miscarriage/spontaneous abortion, sudden infant death syndrome (SIDS), disturbances in neurological development (including hyperactivity, learning difficulties and memory problems), a higher frequency of respiratory infections and below-average lung function (5, 6, 25, 44, 54, 55, 64, 74, 76). Significantly higher risks of preeclampsia (the preliminary stage of eclampsia) and premature birth have been noted in pregnant women who use snuff (30).

Judging from a large number of experiments with laboratory animals, most of these toxic effects can be suspected to be caused directly or indirectly by nicotine (67, 83). A few representative studies and studies with low exposure levels are described below.

Nicotine can have non-specific effects by causing blood vessel constriction, which leads to hypoxia/ischemia, and more specific effects by binding to nicotinic acetylcholine receptors and affecting proliferation and differentiation of nerve cells in both the central and peripheral nervous systems (82, 83). Both the size of the dose and the method of administration affect the toxicity. High single doses (e.g. 2 x 3 mg nicotine/kg b.w./day s.c.), which produce high peaks in plasma, given to pregnant rats for several days, inhibit growth of both mothers and fetuses – in the fetus probably mostly because each injection causes hypoxia/ischemia.

Continuous infusion of 6 mg nicotine/kg b.w./day (yielding a plasma nicotine level of about 84 μg/l, somewhat higher than that of a heavy smoker) has also been shown to inhibit growth without giving visible signs of hypoxia/ischemia such as pale skin or cyanosis (63, 82, 83). With infusions of lower doses, such as 2 mg/kg b.w./day (yielding a plasma nicotine level of about 28 μg/l, equivalent to smoking about one pack of cigarettes per day), no inhibition in growth was observed in either mothers or fetuses (63, 66, 82, 83).

Pregnant mice were given daily s.c. injections of 0.9, 1.8, or 2.7 mg nicotine/kg b.w. (divided into 2 injections per day) during days 0 – 6 of gestation (the first trimester), days 7 – 12 (second trimester) or day 13 –parturition (third trimester). Significant reductions in length of gestation were observed at the lowest and highest doses given in the third trimester (65). These doses roughly correspond to smoking 10, 20 or 30 cigarettes per day.

Rats were given 6.0 mg nicotine/kg b.w. daily (in 2 s.c. injections) on days 4 – 20 of gestation: the treatment resulted in lower growth rates and higher maternal mortality, increased total fetus resorption and lower brain and body weights in young. An increase in ornithine decarboxylase activity in the brain was seen in young, both pre- and postnatal. The effect was most pronounced in the cerebellum and could be observed 20 days after termination of exposure (18 days after parturition). Changes in DNA (synthesis, amount and concentration) were also observed in some parts of the brain: these were also most pronounced in the cerebellum (78). Similar results were obtained with the same dose given by infusion, but the changes were no longer most pronounced in the cerebellum (79). The authors interpret these results as an indication that prenatal nicotine exposure affects early biochemical events that govern nerve cell proliferation and differentiation (78, 79).

In a study in which pregnant rats were exposed by continuous s.c. infusion to 2 or 6 mg nicotine/kg b.w./day on days 5 to 22 of gestation, the high dose caused lower weight gain in the mothers and a small but significant reduction in birth weights of the pups. The day after birth the pups in the high-dose group had much higher mortality after hypoxia provocation (5% 0_2 for 60 or 75 minutes) than either the low-dose group or controls. Further, in comparison with controls, the high-dose group (the low-dose group was not further studied) had lower release of catecholamines from the adrenal medulla during the hypoxia provocation, fewer adrenergic β-receptors in the heart, lower basal noradrenaline metabolism in the brain and a much higher liberation of noradrenaline in the brain after the hypoxia provocation (80).

In a study by Navarro *et al*. (66), pregnant rats were given continuous s.c. infusions of 2 mg nicotine/kg b.w./day from day 4 to day 21 of gestation. The treatment had no effect on either maternal weight or resorption frequency, nor did it affect the pre- or postnatal weight gain of the pups. However, in the brains of the pups (both pre- and postnatal) there was an increase in nicotine binding sites and changes in ornithine decarboxylase activity. Further, DNA in the cerebellum was reduced postnatally, in both concentration and total amount – which the

authors interpret as an indication of a reduced number of cells (66). With nicotine doses of 2 or 6 mg/kg b.w./day administered by the same method on days 4 to 21 (but not if given on days 4 to 12) there was a fairly persistent increase of mRNA that codes for c-fos (a proto-oncoprotein) in the brains of the pups. This increase was seen both prenatally (day 18) and postnatally (on day 2, 3 days after the nicotine infusion was stopped) but without any significant effect on brain weight. The authors discuss the possibility that apoptosis (via activation of c-fos), in addition to inhibiting replication, contributes to the reduction of cells in the central nervous system after prenatal nicotine exposure (81).

Rats were exposed to nicotine by continuous infusion: 0.53, 1.05 or 2.11 mg/kg b.w./day from day 6 or 7 of gestation, resulting in serum nicotine levels of 8, 19 and 35 μg/l respectively. On day 20 the concentrations of nicotine in fetal serum ranged from 91% to 240% of the levels in maternal serum. The ability of the pups (5 or 6 days old) to recover after repeated anoxic provocation was significantly lower for the two higher dose groups (35).

A possible explanation for the weakened defense reaction to hypoxia is provided by Holgert *et al*. (46) in a study of 3-day-old rats. They observed effects of nicotine (0.6 mg/kg b.w., intraperitoneal injections) on peripheral arterial chemoreceptors, which may increase sensitivity to hypoxia and lower the defense reflex in an apnea/hypoxia period. The group later proposed that the β2 subunit in nicotinic acetylcholine receptors (nAChRs) has an important role in the effect of nicotine on parts of the nervous system that govern respiration (both peripheral, on the carotid chemoreceptors, and central, in the brain stem). Mice 35 to 48 days of age had a depressed reaction (lower increase of respiration volume per minute) to hypoxia during sleep after a single i.p. injection of nicotine tartrate (0.5 mg/kg; equivalent to 0.18 mg nicotine/kg b.w.). The nicotine exposure had no effect on mutant mice lacking the β2 subunit of the receptor (22).

Pregnant rhesus monkeys (n = 3) given s.c. infusions of 1.0 mg nicotine/kg b.w./day from day 26 to delivery (by C-section) on day 134 were no lower in weight than controls (n = 3). The concentration of nicotine in amniotic fluid was 15.5 μg/l. The treatment reduced birth weight by 8%, increased the number of nicotine binding sites and increased the expression of nicotinic acetylcholine receptors in the lungs; pulmonary hypoplasia and effects on alveolar development were also observed (76).

Pregnant sheep were exposed to nicotine by continuous s.c. infusion (0.18 mg/kg b.w./day) from day 98 until parturition on day 147 (the third trimester) (41). The exposure yielded a plasma nicotine level of 7 μg/l and a plasma cotinine level of 18 μg/l. Five days after birth, lambs in the nicotine-exposed group (n = 7) had lower respiratory and heart rate responses and slower arousal than controls (n = 11) with hypoxia provocation (10% O_2) during sleep. The authors regard these results as compatible with an effect on peripheral and/or CNS chemoreceptors (41).

Pregnant mice were given s.c. injections of 0.5 mg nicotine/kg b.w./day for 9 or 10 days beginning on day 10 of gestation. Their young had lower postnatal weight

gain (they were followed until day 21), delayed hair growth, eye opening and reflex development, and hyperactivity that persisted until they were mature (3).

Male mice (3, 10 and 19 days old) were given nicotine in s.c. injections of 66 μg/kg b.w., twice a day for 5 days (31). On the day after the final injection, no low-affinity nicotine-binding sites could be detected in cerebral cortex in any of the three groups. In the control groups (given the same treatment with saline solution), 13 to 29% low-affinity nicotine-binding sites were detected. At 4 months of age the mice were examined again for binding sites with low nicotine affinity, spontaneous behavior ("motor activity, locomotion, rearing") and nicotine-induced behavior (provocation with 40 or 80 μg nicotine/kg b.w., s.c.). None of the groups was any different from controls with regard to spontaneous behavior, but the group that was treated on days 10 – 14 still lacked low-affinity nicotine-binding sites and exposure to nicotine resulted in hypoactive behavior. The other two groups were no different from controls with regard to either binding sites or nicotine-induced behavior (increased activity) (31). In similar experiments with rats, lasting effects on nicotine binding in the CNS were seen after treatment with 0.1 mg nicotine/kg b.w. s.c. twice a day on days 8 to 16 after birth. When these animals were compared with controls at the age of 115 days, they had no low-affinity nicotine-binding sites and more high-affinity nicotine-binding sites (62).

In a study by Ankarberg *et al*. (7), groups of 10-day-old male mice were exposed 3.3, 33 or 66 μg nicotine/kg b.w. s.c. twice a day for 5 days. Four months later they were examined for spontaneous and nicotine-induced motor behavior (provocation with 40 or 80 μg nicotine/kg b.w., s.c.). In the two higher dose groups the nicotine exposure elicited hypoactivity, whereas the lowest dose group was no different from controls, i.e. the nicotine caused hyperactivity. Mice in the highest dose group were also tested for learning and memory functions at 4 and 7 months of age. At 4 months there was no difference from controls but at 7 months these skills were significantly lower (7).

Eighteen female Sprague-Dawley rats 25 to 29 days old (6 per group) were given injections of 6.25 ng nicotine/g b.w. (i.p.) at intervals of 6, 8 or 12 hours. For each dose level there were 6 controls given saline solution (18). Twelve hours into the treatment all of them were given (s.c.) 20 IU horse serum gonadotropin to induce follicular development, followed 48 hours later by 10 IU human chorionic gonadotropin (hCG) to induce ovulation. After a further 18 to 20 hours the animals were killed, serum samples were taken, and oviducts were dissected to determine the number of oocytes. There was a dose-dependent reduction in the number of ovulating oocytes, with the LOEL at the lowest dose. S-estradiol concentration was lower in the 2 higher dose groups (no effect at the lowest dose). The study is difficult to interpret, however, since it might have been a local effect on the ovaries. A similar experiment was performed with cotinine but no effects were observed.

Witschi *et al*. (98) showed that nicotine patches applied to pregnant Sprague-Dawley rats, delivering doses of 3.5 mg/day (plasma level 240 μg/l) or 1.75 mg/day (40 μg/l) interrupted pregnancy in 100% and 50% of them, respectively.

Kavitharaj *et al*. (51) report a LOAEL of 0.2 mg/kg for testicular effects on male rats given s.c. injections of nicotine for 21 days. This study, however, is poorly documented.

Dose-response / dose-effect relationships

Dose-effect data for human exposure to nicotine are shown in Table 2.

In addition to exposure from personal use of tobacco, occupational exposure to nicotine occurs via passive smoking, during tobacco cultivation and processing, and in production of pharmaceuticals. These three exposure situations doubtless present a wide variation in absorption kinetics and different effects.

Inhalation of as little as 0.04 mg nicotine can cause local irritation and coughing, and a dose of 0.4 mg affects the heart and circulatory system (42). Systemic effects were observed in rats after i.p. injection of about 0.02 mg/kg (equivalent to 1.4 mg for a human weighing 70 kilos) (18). Effects at such low doses can probably be explained by rapid uptake. It would require high air concentrations of nicotine (> 1 mg/m^3) to achieve the same effect with passive smoking.

Nicotine induces dependency, but the threshold dose for inducing dependency is not known. It has been reported, but not substantiated, that smoking 2 to 5 cigarettes (about 2 to 5 mg nicotine) per day should be habit-forming, and that the rapid and high nicotine intake from inhalation (active smoking) is accompanied by a high risk of dependence. Doses that are associated with addiction in smokers (2 to 5 mg) may not have that effect if uptake is by inhalation over a longer period such as an 8-hour workday.

There are no reports of nicotine dependency initiated by occupational exposure.

For tobacco harvesters, skin uptake from the leaves is predominant and may contribute to the toxic picture (Green Tobacco Sickness). Field workers who harvested the leaves had a median value of 890 μg cotinine/g creatinine (about the same in μg/l) in urine, and 25% of them had symptoms. This cotinine concentration in urine corresponds to an intake of about 10 mg nicotine/day (≈ 8 to 10 cigarettes per day). Assuming a 50% uptake and an inhaled air volume of 10 m^3, this corresponds to an air concentration of about 2 mg/m^3.

In pharmaceutical manufacture it is again inhalation exposure that seems to be most important (if adequate protective clothing is worn). Production conditions are probably reflected best in the animal experiments that report an effect in the form of weight loss at an air concentration of 0.5 mg/m^3 (see below). High air concentrations of nicotine compounds in powder form irritate airways (coughing).

In several studies pertinent in this context, exposure levels were calculated from metabolites in urine or plasma. There is a simple formula that can be used to calculate the dose. Where skin uptake may be relevant, urine and blood will usually provide a more accurate dose estimate than air concentration.

Rats were exposed by inhalation to 0.5 mg/m^3 nicotine, 20 hours/day, 5 days/week for two years (plasma nicotine 100 μg/l, about twice that of a smoker). There was no elevation in mortality or arteriosclerosis, although these rats had lower weights than controls (88).

Nicotine has been shown to have toxic effects on reproduction in laboratory animals (see Table 3). Rats given high doses of nicotine (6 mg/kg b.w./day, s.c.) during gestation have higher frequencies of fetal absorption and bear pups with lower birth weights and lower postnatal growth rates. A maternal dose of 2 mg nicotine/kg b.w./day (equivalent to smoking a pack of cigarettes a day) resulted in an increase in nicotine binding sites, changes in ornithine decarboxylase activity and reduction of the amount of DNA in the central nervous systems of the young. A depressed response to hypoxia/anoxia was observed in the young of rats exposed to 1.05 mg nicotine/kg b.w./day (plasma nicotine level 19 μg/l) and in lambs when their mothers had been exposed to 0.18 mg nicotine/kg b.w./day (plasma nicotine 7 μg/l, plasma cotinine 18 μg/l) during gestation. Effects on lung development have been observed in the young of rhesus monkeys exposed to 1.0 mg nicotine/kg b.w./day during gestation. Delayed hair growth, eye opening and reflex development, lower postnatal weight gain and hyperactivity persisting until maturity were observed in the young of mice exposed to 0.5 mg nicotine/kg b.w./day during gestation. Lack of low-affinity nicotine-binding sites was observed in the CNS of adult rats and mice treated with 200 or 132 μg nicotine/kg b.w./day, respectively, from birth until about 2 weeks of age. Effects on memory and learning functions were observed in 7-month-old mice treated from 10 to 15 days of age with 132 μg nicotine/kg b.w./day (s.c.), and at a dose of 66 μg there were changes in nicotine-induced motor behavior at 4 months of age. No effect was observed at 7 μg. A dose of 66 μg/kg b.w./day given to mice (LOAEL) is equivalent for humans to inhalation exposure to an air concentration of 0.13 mg/m^3, assuming a body weight of 70 kg, a caloric factor of 7 (52), 50% absorption in the lungs, and an inhaled air volume of 10 m^3 during 8 hours. Using the same calculation for a dose of 7 μg/kg (the NOAEL) yields an air concentration of 0.014 mg/m^3.

Conclusions

Available scientific material is not sufficient to identify a critical effect of occupational exposure to nicotine. Judging from animal experiments, the critical effect of nicotine exposure is its effect on reproduction, with effects on the development of the nervous system in the young. Changes in nicotine-induced motor behavior have been observed in 4-month-old mice treated from 10 to 15 days of age with 66 μg nicotine/kg b.w./day (divided into 2 s.c. injections/day). Recalculated to job-related inhalation exposure, this dose correspond to an air level of about 0.1 mg nicotine/m^3.

Symptoms of acute poisoning – Green Tobacco Sickness, with dizziness, vomiting, headache, weakness, stomach pain, cramps, salivation, sweating – are

common among tobacco harvesters, where nicotine exposure is primarily via skin uptake. The symptoms have been observed at cotinine levels of about 900 μg/l in urine (median), which corresponds to an inhalation exposure of about 2 mg/m^3.

Nicotine is addictive, but the threshold dose for the addictive effect is not known.

Nicotine exposure equivalent to smoking also has acute cardiovascular effects.

Animal experiments have not shown that nicotine is carcinogenic. There are no carcinogenicity studies of humans exposed to nicotine alone.

Nicotine is easily absorbed through the skin, and skin exposure may lead to acute poisoning with severe outcome.

Table 2. Dose-effect / dose-response relationships observed in studies in which human subjects were exposed to nicotine.

Exposed groups, study design	Urine cotinine (μg/l)	Effects	Ref.
Tobacco harvesters, non-users of tobacco, good protective clothing N = 10; 5 controls	100 (median)	No symptoms	24
Tobacco harvesters, non-users of tobacco, poor protective clothing N = 43	0 – 24 pre-shift 26 - 2930 (median 890) next morning	25% had Green Tobacco Sickness symptoms: dizziness, vomiting, headache, weakness, stomach pain, cramps, excessive salivation and perspiration	39
Tobacco harvesters, poor control of tobacco use, poor protective clothing N = 289	3800	53% with GTS symptoms	40
Three groups: snuff users, smokers, no tobacco use Prospective cohort study 12 years N = 135,036	- *	Elevated mortality from heart and circulatory diseases for snuff users (RR = 1.4, 95% CI 1.2-1.6) and smokers (RR = 1.9, 95% CI 1.7-2.2); no increase of cancer deaths for snuff users (RR =1.1, 95% CI 0.9- 1.4) RR for smokers 2.5 (95% CI 2.2-3.0)	19
Two groups: snuff users/no tobacco use Case-control study N = 585/589	- *	No elevation in risk for heart infarct for snuff users (RR = 0.89, 95% CI 0.62 - 1.29)	47
Healthy volunteers 21 inhalations of 0.01 ml nicotine aerosol, concentration interval 0 - 64 mg/ml N = 24	-	Elevated blood pressure and heart rate, dose-dependent increase of airway resistance, coughing attacks. LOAEL 2 mg/ml; 0.4 mg	42

* Smokers and snuff users have plasma nicotine levels around 20 to 30 μg/l and urine cotinine around 2000 - 3000 μg/l.

Table 3. Effects of nicotine exposure on reproduction of laboratory animals.

Dose (mg/kg/day), Exposure method	Species	Exposure time	Plasma nicotine (μg/l)	Effects	Ref.
6, infusion	Rat	prenatal	84	Growth inhibition	63, 82, 83
2, infusion	Rat	prenatal, days 4-21	28	Reduced concentrations and amounts of DNA, changes in ornithine decarboxylase activity, increase of nicotine-binding sites and mRNA coding for c-fos in CNS	63, 66, 81, 82, 83
2.7, injection*	Mouse	prenatal, day 13 to birth (day 19)		Shortened gestation	65
1.8, injection*	Mouse	prenatal, day 13 to birth (day 19)		--	
0.9, injection*	Mouse	prenatal, day 13 to birth (day 19)		Shortened gestation	
2.11, infusion	Rat	prenatal, day 6-7 to birth	35	Impaired recovery after anoxia	35
1.05, infusion	Rat	prenatal, day 6-7 to birth	19	Impaired recovery after anoxia	
0.53, infusion	Rat	prenatal, day 6-7 to birth	8	No observed effect	
1.0, infusion	Rhesus monkey	prenatal, day 26 to C-section on day 134	**	Effects on lung development. Increase of nicotine-binding sites and receptors in lungs	76
0.5, injection	Mouse	prenatal, day 10 to day 19 - 20		Lower postnatal weight gain, delayed hair growth, eye-opening and reflex development; hyperactivity persisting to maturity	3
0.2, injection*	Rat	postnatal, days 8-16		At 115 days, lack of low-affinity nicotine-binding sites and increased number with high affinity	62
0.18, infusion	Sheep	prenatal, day 98 to birth (day 147)	7 ***	Weakened arousal with hypoxia	41
0.132, injection*	Mouse	postnatal, days 10 to 14		At 4 months, lack of low-affinity nicotine-binding sites and hypoactive behavior with nicotine provocation	31
0.132, injection*	Mouse	postnatal, days 10 to 14		Lower learning and memory at 7 months	7
0.066, injection*	Mouse	postnatal, days 10 to 14		Hypoactivity with nicotine provocation at 4 months (LOAEL)	
0.007, injection*	Mouse	postnatal, days 10 to 14		No effect (NOAEL)	

*divided into 2 injections/day
**15.5 μg nicotine/l in amniotic fluid
***18 μg cotinine/l in plasma

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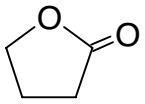
Consensus Report for γ-butyrolactone

June 2, 2004

This Report is based primarily on a criteria document compiled by the Nordic Expert Group (28).

Chemical and physical data

CAS No.	96-48-0
Synonyms:	dihydro-2(3-H)-furanone; 4-butyrolactone; tetrahydro-2-furanone; 1,2-butanolide; 1,4-butanolide; 4-hydroxybutyric acid lactone
Formula:	$C_4H_6O_2$
Structure:	
Molecular weight:	86.1
Boiling point:	206 °C
Melting point:	- 44 °C
Flash point:	98 °C (open cup)
Vapor pressure:	0.15 kPa (20 °C)
Saturation concentration:	1480 ppm (20 °C)
Water solubility:	mixes with water
pH:	4.51 (10% in water)
Conversion factors:	1 ppm = 3.57 mg/m^3 (20 °C) 1 mg/m^3 = 0.28 ppm (20 °C)

γ-Butyrolactone (GBL) is a colorless, oily liquid with relatively low vapor pressure and a mild, caramel-like odor. It is soluble in e.g. methanol, ethanol, acetone and benzene, and mixes with water. There is a pH-dependent equilibrium between GBL and γ-hydroxybutyrate (GHB). In basic media GBL is hydrolyzed quite rapidly to GHB, but in acidic media the hydrolysis is slow. Complete transformation to GHB occurs within a few minutes at pH 12, whereas an equilibrium mixture of 2:1 GBL:GHB occurs after months in pure water and within days at pH 2 (28).

Occurrence and use

GBL occurs in nature, and has been identified in some foods (e.g. meat, tomatoes, coffee) and in alcoholic drinks (28). GBL is used as a solvent, in paint removers, as a pH regulator, in vulcanizing, to modify viscosity, in etching, and as a chemical intermediate in production of pyrrolidones, herbicides/pesticides and medicines (28). Use of GBL as a dietary supplement, tranquilizer and treatment for alcoholism has also been reported. GBL is also used as a "party drug" (1, 5).

In Sweden, GBL in pure form is used primarily as a solvent in the electronics industry and in manufacturing of various products, and the major products containing GBL are paint removers (graffiti removers), cleaners, paints, electrolytes and developers (20, 23).

In other countries GHB has been used as an anesthetic and tranquilizer and in treatment of narcolepsy, alcoholism and opiate addiction. The substance has also appeared as a dietary supplement used by body-builders and dieters. GHB is also used as a recreational drug, and in recent years has been classified as a narcotic in several countries, including Sweden (9, 23)

Uptake, biotransformation, excretion

GBL can be absorbed via the skin. The reported absorption rate for human skin *in vitro* is 110 μg/cm^2/hour at steady state (32). Using this absorption rate and applying the ECETOC criterion for skin notation (8), i.e. exposure of 2000 cm^2 skin (equivalent to hands and lower arms) for 1 hour, yields a skin uptake equal to about 25% of uptake via inhalation exposure to 50 ppm for 8 hours (the provisional Danish threshold limit for GBL), assuming inhalation of 10 m^3 air and 50% uptake of GBL. When GBL was applied to the skin of rats (546 mg/kg b.w.), the highest GHB concentrations in plasma were seen after 0.5 to 2 hours. At least 10% of the applied dose of GBL was absorbed (11). Uptake of GBL via the digestive tract is rapid and complete (28). GBL is absorbed from the digestive tract more rapidly, and yields higher plasma levels, than GHB (5, 15, 19, 26). When rats were given GBL in oral doses of 136 or 546 mg/kg b.w., plasma concentrations of GHB peaked within 1 hour (11, 19). No data on inhalation uptake were found (28).

GBL is transformed in blood and liver to the neurologically active metabolite GHB within a few minutes. This hydrolysis is catalyzed by the enzyme lactonase. GHB also occurs naturally in micromolar concentrations in the brain and peripheral tissues (9, 23, 28). GHB passes through the blood-brain barrier and the placental barrier (9). GBL is more fat-soluble than GHB and has a different distribution pattern – producing, for example, higher concentrations in the brain. Thus, despite the rapid hydrolysis of GBL in blood, there is some accumulation in various tissues of this precursor to GHB (15, 19). Metabolism of GHB is not completely understood, though it is known to vary with plasma level and the organ involved (28). The average half time for GHB in rat blood after intravenous

administration of GBL (500 mg/kg b.w.) is reported to be about 45 minutes (28). Elevated excretion of glycolate, *S*-3,4-dihydroxybutyrate and other substances has been observed in human urine after oral administration of GBL, which suggests metabolism via β-oxidation. Acetyl-CoA, and ultimately CO_2 (via the citric acid cycle) are formed via this metabolic pathway (18, 28). In an alternative metabolic pathway, GBL is oxidized to succinate, which enters the citric acid cycle (1). Metabolization from GHB to γ-aminobutyrate (GABA) has also been reported (33). When rats were given a single intravenous injection of ^{14}C-labeled GHB, 60% of the radioactivity was excreted as $^{14}CO_2$ within 2.5 hours (28). Similar results were obtained with GBL. GBL is thus eliminated primarily as metabolites in urine and as CO_2 via the lungs (28).

Toxic effects

Human Data

No reports on effects of occupational exposure were found. Several cases of poisoning due to oral intake of GBL or GHB for recreational purposes have been described, however. Symptoms and clinical findings after intake of GBL and GHB are similar, although it is not clear whether repeated use can result in permanent damage (23, 28). Acute toxic effects include bradycardia, hypothermia, CNS depression, loss of consciousness (usually for 1 or 2 hours), confusion, aggressiveness, and uncontrolled movements (28). There are large differences in the nature and degree of symptoms shown by different individuals, however (9). One patient with symptoms of poisoning is reported to have taken about 90 mg GBL/kg b.w. (negative blood ethanol test) (5). In another study, unconsciousness is reported in 2 men who drank 50 ml of a nail polish remover containing 50% GBL and 50% ethanol. Bradycardia was detected and treated, and the patients recovered after a couple of hours (2).

Pulmonary edema and acute effects on the nervous system with coma were observed in a child who had drunk (and probably also inhaled) a product containing GBL (24). Chronic use of GBL can lead to addiction and neurotoxic effects including anxiety, depression and tremor (13).

In general, a single oral dose of about 1 to 4 grams (≈ 15 – 60 mg/kg b.w.) is necessary to achieve a sedative effect in humans (23). Clinical experience has shown that GBL is more effective than GHB in putting patients to sleep (19). GHB is reported to cause short-term memory loss and hypotonia at an oral dose of 10 mg/kg b.w., euphoria at 20 – 30 mg/kg, anesthesia at 50 mg/kg, and coma at 50 – 70 mg/kg (4, 9, 23, 28). An intravenous dose of 50 to 60 mg/kg b.w. causes general anesthesia within 5 minutes (9). Poisoning with GHB is usually a result of using the substance as a "party drug" (often together with alcohol) at oral doses of around 2 to 3 g (about 35 mg/kg b.w.). The CNS effects are potentiated by simultaneous intake of alcohol. A lethal dose of GHB alone can be roughly estimated to be about 20 to 30 grams (≈ 300 – 400 mg/kg b.w.) or higher (9, 23).

Animal data

GBL has moderate to low acute toxicity to laboratory animals. Animal data also show that GBL is more potent than GHB with oral as well as parenteral administration (19). The toxic picture is characterized by CNS effects with anesthesia. In rats, a biphasic effect has been observed at relatively low doses of GBL, with an initial reduction in activity followed by hyperactivity (21, 28). The LD_{50} for GBL has been reported to be 5600 mg/kg b.w. for skin application to guinea pigs, and 500 – 1800 mg/kg b.w. for oral administration to rats, mice and guinea pigs (28). Temporary changes in EEG and behavior (including immobility) have been reported in juvenile rats after a single intraperitoneal injection of 50 mg GBL/kg b.w., and in adult rats at 150 mg/kg b.w. (29). In tests measuring spontaneous activity and coordination, a temporary decline was observed in mice after a single intraperitoneal injection of 55 mg GBL/kg b.w.; the effect was also noticeable at 22 mg/kg b.w. (27). In an unpublished study with rats reversible effects including lassitude, shallow breathing and clear nasal secretion, but no deaths, are reported with 4 hours of inhalation exposure to a dose level of 5100 mg GBL/m^3 (28). In another unpublished rat study, the LC_{50} for 4 hours of exposure is reported to be >2680 mg/m^3 (10).

Mice and rats were given GBL by gavage on 12 days during a 16-day period. Doses were 0, 87, 175, 350, 700 or 1400 mg/kg b.w./day for mice and 0, 75, 150, 300, 600 or 1200 mg/kg b.w./day for rats. Immediately after the dosing, inactivity, and in some animals irregular respiration/breathlessness, was observed in the mice at doses of 350 mg/kg and above, and in the rats at 600 mg/kg and above. Lower weight gain was noted in the female rats given 600 mg/kg b.w. Nearly all the rats and mice given the highest dose died (1200 and 1400 mg/kg b.w., respectively) (21, 28).

In a 13-week study, mice and rats were given GBL by gavage 5 days/week. Doses were 0, 65, 131, 262, 525, or 1050 mg/kg b.w. for the mice and 0, 56, 112, 225, 450 or 900 mg/kg b.w. for the rats. No noteworthy histopathological changes were observed. Inflammation in nasal mucosa was seen in the rats, but was attributed to reflux of GBL during the dosing. Temporary immobility after administration was seen in all the rats given 900 mg/kg, and during week 8 all the males and one female in this dose group died. During the first 2 to 3 weeks, the rats receiving 225 or 450 mg/kg had lower activity levels immediately after dosing, but then they seemed to develop tolerance. Male rats receiving 450 mg/kg also had lower weight gain. GBL-related deaths were also seen in mice given the highest dose (1050 mg/kg b.w./day). Males in this dose group also had lower growth. Moderate inactivity after dosing was seen in mice given 262 mg/kg, and at higher doses the mice were inactive for several minutes. These acute reactions, however, faded after 3 – 4 weeks in groups receiving 525 mg/kg or less (21, 28). It is reported in a cancer study (see below, Mutagenicity, carcinogenicity) that female rats given oral doses of 450 mg GBL/kg b.w./day, 5 days/week for 2 years, had lower growth but showed no indications of toxicity. No noteworthy effects were reported in either sex at 225 mg/kg b.w./day. Inhibited growth was also

observed in male and female mice given 262 or 525 GBL/kg b.w. on the same schedule, and in the high-dose groups (525 mg/kg/day) there were indications of CNS effects shortly after the dosing. Elevated mortality was also reported for male mice in the high-dose group (aggression and stress-related effects) (21, 28).

No acceptable studies of skin irritation were found, making it difficult to draw any clear conclusions. However, there are some older data indicating that GBL can have a weak skin-irritating effect (6, 28). GBL has also been reported to cause eye irritation, but not lasting eye damage, to laboratory animals (28). In older, unpublished studies, instillation of GBL is reported to cause severe irritation to conjunctiva and damage to cornea, iris and conjunctiva (6, 28). In more recent studies, GBL has been described as irritating to eyes *in vivo* as well as *in vitro* (12, 28). According to an estimate based on structure-activity relationship, GHB, but not GBL, should be expected to irritate the eyes. "Respiratory hypersensitivity" was also predicted for GHB but not GBL (25).

Mutagenicity, carcinogenicity

GBL has been studied in many *in vitro* tests and some *in vivo* tests. It is not mutagenic in bacterial tests and has not been found to be mutagenic or genotoxic in tests on yeasts (28).

GBL also yielded negative results in several *in vitro* test systems for primary DNA damage. Regarding tests on mammalian cells *in vitro*, chromosome aberrations and sister chromatid exchanges were observed in one study with high concentrations of GBL (and addition of exogenic metabolizing systems), but were not observed in two other studies with lower concentrations. Negative results were also reported in *in vitro* gene mutation tests. Oncogenic transformation of mammalian cells was observed in one of two studies, but this test has been judged to be of little value in predicting carcinogenicity. In tests with *Drosophila*, neither oral administration of up to 2.8% in feed nor injection of 1.5% GBL was observed to cause genetic mutations or recombinations. Nor was any increase in micronuclei observed in bone marrow cells of mice given intraperitoneal injections of GBL (2 x 560 or 2 x 984 mg/kg b.w.) (21, 28). GBL was also negative in a test measuring mutagenicity in mouse germ cells (100 – 400 mg/kg b.w., i.p.) and in a sperm morphology assay (5 x 0.1 – 1 mg/kg b.w./day, i.p.) (22, 31). One work reports structure-activity predictions indicating that GBL may be active in *in vivo* tests for SCE and micronuclei. The overall assessment, however, is that GBL was probably not genotoxic (25).

In an NTP study, GBL was given by gavage to rats and mice 5 days/week for up to 2 years. Doses were 0, 112 or 225 mg/kg b.w. for male rats, 0, 225, or 450 mg/kg b.w. for female rats, and 0, 262 or 525 mg/kg b.w. for the mice. There was no evidence of carcinogenic activity in either the rats or the female mice. However, in the female rats there were lower incidences of cysts and fibroadenomas in mammary glands and of pituitary cysts. The results for male mice were difficult to interpret, largely because of the low survival rate in the high-

dose group. An elevated incidence of proliferative damage in the adrenal medulla, especially focal hyperplasia, was noted in male mice in the low-dose group. A reduced incidence of hepatic-cell tumors was also observed in the male mice (21, 28). In other studies no increase in tumor incidence definitely attributable to GBL has been seen in mice or rats given GBL by oral administration, skin application or subcutaneous injection (28).

Assessments based on chemical structure and genotoxicity and toxicity tests indicate that it is unlikely that GBL has a carcinogenic effect (14, 25, 30). The IARC assessment published in 1999 stated that it could not be determined whether GBL is carcinogenic to humans, but that data indicate it is not carcinogenic to experimental animals. GBL was therefore placed in Group 3: "not classifiable as to its carcinogenicity to humans" (28).

Effects on reproduction

In a study in which rats were given single i.p. injections of GBL in doses ranging from 62.5 to 750 mg/kg b.w., a significant reduction of luteinizing hormone (LH) in serum was observed at doses of 250 mg/kg and higher. Reduction of follicle-stimulating hormone (FSH) in serum was observed at 500 mg/kg (anesthetic dose) and higher. A reduction in the number of ovulating rats was noted at all dose levels, and at 750 mg/kg (anesthetic dose) ovulation was blocked in all animals. At this level uterus weight was also significantly elevated (3). A direct effect on oocytes, resulting in inhibited maturity, was indicated in an *in vitro* study with GBL (17).

In an incompletely reported study, greatly reduced testes weights were observed in prepubertal rats given 0.5% or 1% GBL in drinking water (≈ 550 or 1100 mg/kg b.w./day), probably for 20 days. There was no observed difference in serum prolactin levels between treated animals and controls (7, 28).

Rats were given GBL by gavage in doses of 10, 50, 125, 250 or 500 mg/kg b.w./day on days 6 to 15 of gestation: somewhat reduced placental weights were reported at all dose levels and significantly higher fetal weight in some dose groups (50 – 250 mg/kg/day). No other exposure-related effects were observed in either embryos or fetuses, and there were no significant differences in pre- or post-implantation losses (16).

Dose-effect / dose-response relationships

There are no data that can be used directly to estimate a dose-effect or dose-response relationship for occupational exposure to GBL. Dose-effect relationships for oral administration of GBL are not well studied either, but one study reports that a patient with symptoms of poisoning had taken about 90 mg GBL/kg b.w. (5). GBL is transformed rapidly in the body to the neurologically active metabolite GHB, and symptoms and clinical observations of patients after intake of either substance are about the same (23, 28). However, there are quantitative differences in uptake and metabolism. GBL is taken up from the digestive system

more rapidly and produces higher plasma levels than GHB. GBL is also more fat-soluble than GHB, and the distribution pattern is different: GBL yields higher concentrations in the brain, for example (19). Both clinical experience and animal experiments also show that GBL is more potent than GHB with both oral and parenteral administration (19). GHB given orally is reported to cause short-term memory loss and hypotonia at 10 mg/kg b.w., euphoria at 20 – 30 mg/kg, anesthesia at 50 mg/kg and coma at 50 – 70 mg/kg (4, 9, 23, 28). An oral dose of about 1 to 4 g (≈ 15 – 60 mg/kg b.w.) is usually required to achieve a tranquilizing or sedating effect in humans (23). A lethal dose of GHB can be roughly estimated to be around 20 – 30 g (300 – 400 mg/kg b.w.) or more (9, 23).

Using dose-effect data for oral doses of GHB, the following calculation can be made. Acute CNS effect on humans after oral intake of GHB have been reported at about 10 to 50 mg/kg body weight. Recalculating to an 8-hour inhalation exposure, and assuming 100% uptake, 70 kg body weight and 10 m^3 inhaled air, yields a level of 70 to 350 mg GHB/m^3 (16 to 81 ppm). Extrapolating in this manner from oral to inhalation exposure, however, tends to overestimate the risk of inhalation, since CNS effects are probably related to the peak level of GHB in plasma, and oral doses yield a higher peak than inhalation of the same total dose over an 8-hour period.

Taking the dose-effect relationships for oral administration of GHB and re-calculating for GBL, the inhalation level for GBL would be 58 – 290 mg/m^3 (16 – 81 ppm). Since GBL seems to be more toxic than GHB, however, this calculation probably provides an underestimate of the risk.

Dose-effect relationships observed in laboratory animals treated with GBL are shown in Table 1.

Conclusions

There are no data on which to establish a critical effect of occupational exposure to GBL. Experience with GBL and its metabolite GHB from cases of oral intake for the purpose of becoming intoxicated, cases of poisoning and animal data all indicate that the critical effect of GHB is its acute effects on the central nervous system. Limited animal data indicate that GBL may affect fertility. GBL in direct contact with eyes can cause eye irritation. Skin exposure to GBL in liquid form can result in significant systemic exposure.

Table 1. Effects of GBL on mice and rats. (i.p. = intraperitoneal, p.o. = per os)

Exposure	Species	Effects	Ref.
22 mg/kg bw single dose, i.p.	Mouse	Temporary reduction in mobility and coordination	27
50 mg/kg bw/day days 6-15 of gestation, p.o.	Rat	Increased fetal weight, somewhat lower placental weight	16
50 mg/kg bw single dose, i.p.	Rat (juvenile)	Temporary EEG changes and effects on behavior (including inactivity)	29
55 mg/kg bw single dose, i.p.	Mouse	Temporary declines in activity and coordination	27
62.5 mg/kg bw single dose, i.p.	Rat	Inhibited ovulation in 22% of animals	3
125 mg/kg bw single dose, i.p.	Rat	Inhibited ovulation in 20% of animals	3
150 mg/kg bw single dose, i.p.	Rat	Temporary EEG changes and effects on behavior (including inactivity)	29
175 mg/kg bw/day 12 days, p.o.	Mouse	NOAEL	21
225 mg/kg bw/day 5 days/week, 13 weeks, p.o.	Rat	Slight inactivity after dosing during the first few weeks	21
250 mg/kg bw single dose, i.p.	Rat	Inhibited ovulation in 63% of animals, reduction of LH in serum	3
262 mg/kg bw/day 5 days/week, 13 weeks, p.o.	Mouse	Moderate inactivity after dosing during the first few weeks	21
262 mg/kg bw/day 5 days/week 2 years, p.o.	Mouse	Inhibited growth Males: elevated incidence of proliferative damage in the adrenal medulla	21
300 mg/kg bw/day 12 days, p.o.	Rat	NOAEL	21
350 mg/kg bw/day 12 days, p.o.	Mouse	Inactivity after dosing, irregular respiration	21
450 mg/kg bw/day 5 days/week 13 weeks, p.o.	Rat	Slight inactivity after dosing during the first few weeks Males: lower weight gain	21
450 mg/kg bw/day 5 days/week, 2 years, p.o.	Rat (females)	Inhibited growth	21
500 mg/kg bw single dose, i.p.	Rat	Anesthetic effect, inhibited ovulation in 71% of animals, reduction of LH and FSH in serum	3
0.5% in drinking water, (≈550 mg/kg/day) probably 20 days	Rat	40% reduction in testes weight	7

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Summary

Montelius J (ed). *Scientific Basis for Swedish Occupational Standards*. XXV. Arbete och Hälsa 2005:7, pp 1-123. National Institute for Working Life, Stockholm.

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 National Institute for Working Life from July, 2003 through June, 2004.

Key Words: Cobalt, Cobalt compounds, γ-Butyrolactone, GBL, 4,4'-Methylene-bis(2-chloroaniline, MOCA, Nicotine, Occupational exposure limit (OEL), Risk assessment, Scientific basis, SMF, Synthetic mineral fibers, Tin, Tin compounds, Toxicology.

Sammanfattning

Montelius J (ed). *Vetenskapligt underlag för hygieniska gränsvärden*. XXV. Arbete och Hälsa 2005:7, s 1-123. Arbetslivsinstitutet, Stockholm.

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 2003 - juni 2004.

Nyckelord: γ-Butyrolakton, 4,4´-Diamino-3,3´-diklorofenylmetan, GBL, Hygieniskt gränsvärde, Kobolt, Koboltföreningar, MOCA, Nikotin, Riskvärdering, SMF, Syntetiska oorganiska fibrer, Tenn, Tennföreningar, Toxikologi, Vetenskapligt underlag.

En svensk version av dessa vetenskapliga underlag finns publicerad i Arbete och Hälsa 2004:16.

APPENDIX

Consensus reports in this and previous volumes

Substance	Consensus date	Volume in Arbete och Hälsa	(No.)
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, C_{10}-C_{15}	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)
p-Aminoazobenzene	February 29, 1980	1981:21	(I)
Ammonia	April 28, 1987	1987:39	(VIII)
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 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)
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, C_5-C_{15}	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)
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)
reviderat	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 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)
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)
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-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)
Hydrogen bromide	February 11, 1998	1998:25	(XIX)
Hydrogen cyanide	February 7 2001	2001:20	(XXII)
Hydrogen fluoride	April 25, 1984	1984:44	(V)
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)
Lead, inorganic	February 29, 1980	1981:21	(I)
revised	September 5, 1990	1992:6	(XII)
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)
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)
Nitroethane	April 4,	1989	1989:32	(X)
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)
Oil mist	April 8,	1981	1982:9	(II)
Organic acid anhydrides, some	September 12,	1989	1991:8	(XI)
Oxalic acid	February 24,	1988	1988:32	(IX)
Ozone	April 28,	1987	1987:39	(VIII)
Paper dust	February 7,	1990	1991:8	(XI)
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)
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 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 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)
Sulfur dioxide	April 25, 1985	1985:32	(VI)
Sulfur fluorides	March 28, 1990	1991:8	(XI)
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)
Wood dust	June 17, 1981	1982:9	(II)
revised	June 25, 2000	2000:22	(XXI)
Xylene	February 29, 1980	1981:21	(I)
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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