NR 2001:20

Scientific Basis for Swedish Occupational Standards XXII

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ARBETE OCH HÄLSA | VETENSKAPLIG SKRIFTSERIE
ISBN 91-7045-624-0 ISSN 0346-7821 http://www.niwl.se/

Arbetslivsinstitutet
National Institute for Working Life

Arbete och Hälsa

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Summaries in Swedish and English as well as the complete original text are available at www.niwl.se/ as from 1997.

National Institute for Working Life
S-112 79 Stockholm
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ISBN 91–7045–624–0
ISSN 0346–7821
http://www.niwl.se/
Printed at CM Gruppen, Bromma

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 data bases 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. 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 22nd volume which is published and it contains consensus reports approved by the Criteria Group during the period July 2000 to June 2001. These and previously published consensus reports are listed in the Appendix (p 90). Technical editing for printing was made by Karin Sundström.

Johan Högberg
Chairman

Johan Montelius
Secretary

The Criteria Group has the following membership (as of June, 2001)

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Margareta Littorin, Staffan Skerfving, Department of Occupational and Environmental Medicine, University Hospital, Lund (the section "Biological measures of exposure").

Consensus Report for Ethylenethiourea

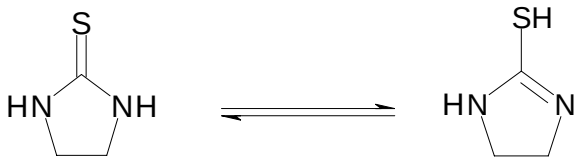
September 27, 2000

This Consensus Report is based largely on a criteria document from the Dutch Expert Committee for Occupational Standards (DECOS) (15), and takes into account research published through 1999. The last literature search was made in May, 2000.

Chemical and physical data

CAS No.:	96-45-7
Synonyms:	ETU
	imidazoline-2-thiol
	2-imidazolidinethione
	2-mercaptoimidazoline
Formula:	$C_3H_6N_2S$

Structure:



Molecular weight:	102.15
Melting point:	203-204 °C
Relative density:	1.4 (water = 1)
Vapor pressure:	0.0027 hPa (100 °C) (3)
Solubility:	in water: 20 g/liter (30 °C)
	in ethanol: moderate
	in acetone, ether, chloroform: insoluble

Ethylenethiourea (ETU) at room temperature is a white to pale green, crystalline powder with a weak amine-like odor and bitter taste. ETU is resistant to hydrolysis, but is readily oxidized in biological systems and on exposure to air and light.

Occurrence, use

Occupational exposure to ETU may occur in the rubber industry, where it is used for vulcanization of polyacrylate rubber and as an accelerator in the manufacture of neoprene rubber. ETU has also been used in production of antioxidants and synthetic resins. Exposure to ETU may also occur in forestry and agriculture, where metallic salts of ethylenebisdithiocarbamate (e.g. maneb, mancozeb, zineb) are used as fungicides. These products usually contain ETU as an impurity. ETU is formed in biological systems by the breakdown of ethylenebisdithiocarbamate.

ETU can be synthesized by a reaction between ethylene diamine and carbon disulfide, followed by addition of hydrochloric acid to close the ring.

ETU does not occur naturally in the environment. Non-occupational exposure in Poland was estimated by measuring the concentration of ETU in various foods, and intake was found to be between 0.01 and 1 µg/kg body weight/day (33). In the populations of four Italian towns, measured excretion of ETU in urine was in the range <0.1 to 8.3 µg/g creatinine (5). In a wine district where ethylenebisdithiocarbamate was used as a fungicide, excretion of ETU in urine was higher: up to 61.4 µg/g creatinine. The highest values were found in smokers and wine drinkers (5). In a laboratory study with five volunteers in which the amount of ETU in diet was determined by analysis, it was found that most of the ETU in urine originated from intake of wine (4). ETU has been found in tobacco smoke (8 to 27 ng/cigarette in 4 of 12 tested brands) (7). FAO/WHO have proposed 4 µg/kg body weight/day as an acceptable intake of ETU (19). The EU threshold limit value for ETU in foods is 50 µg/kg (cited in Reference 17).

In a Finnish study of groups of agricultural and forestry workers who used mancozeb or maneb fungicides (containing ethylenebisdithiocarbamate), air concentrations of ETU around spraying ranged from 0.14 to 0.6 µg/m^3 (average values within the groups). Air levels were higher around weighing (highest average value 1.81 µg/m^3). The highest concentration of ETU measured in urine was 23 µg/liter (49). Another Finnish study of 29 potato farmers (probably including at least some of the participants in the previously mentioned study) showed air concentrations of 0.004 to 3.3 µg/m^3 in the breathing zone and 0.006 to 0.8 µg/m^3 in the tractor cab. In the 24 hours immediately after the exposure, excretion of ETU in urine was in the range 0.09–2.5 µg/mmol creatinine (0.8-22.1 µg/g creatinine) (52). In 1980, air concentrations of 120 to 160 µg/m^3 were measured in an English rubber factory where ETU was used in a process that generated dust (84). In an ETU production plant in England, air levels shown on personal monitors were 10 to 240 µg/m^3, with a single reading of 330 µg/m^3 (84).

Uptake, distribution, excretion

Data from animal experiments show that ETU is rapidly absorbed from the digestive tract. IPCS/WHO report that ETU was identified in the blood of rats as early as 5 minutes after oral administration of ^{14}C-ETU (100 mg/kg body weight)

(33). A study with guinea pigs (reviewed in Reference 3) showed that uptake of ETU through intact skin was relatively slow: 14% of 2-^{14}C-ETU (15 mg/ml, 1 ml applied to an area of 4 x 4 cm) was absorbed within 24 hours. If the skin was damaged, uptake within 24 hours was 42%. The only laboratory data indicating respiratory uptake of ETU are in unpublished studies on rats (3). There are no quantitative data on ETU uptake by humans, although the substance has been found in urine of occupationally exposed persons (49, 52). It was found that ETU in the urine of workers producing fungicides was correlated to the amounts of mancozeb and ETU on their hands (6). It was concluded that most of the ETU exposure in this work environment was due to skin uptake (6).

Regardless of the path of absorption, ETU accumulates in the thyroid (15). Single doses of ETU (20 mg/kg body weight) were given to rats and guinea pigs by gavage, and 96 hours later there was a much higher accumulation of ETU in thyroid than in liver, kidney, heart and muscle tissue, where concentrations were about the same (64). When 2-^{14}C-ETU was given to pregnant rats, the radioactivity was evenly distributed in all examined tissues except the thyroid, where accumulation was particularly marked after 24 hours (>30 times). After 2 and 6 hours, the concentration in the thyroid was two to three times higher than in other tissues. The concentration of ETU was somewhat lower in fetal tissue than in the mothers. This study also shows that ETU can cross the placental barrier (38). Rhesus monkeys (2 females) were given single oral doses of ETU (40 mg/kg body weight) and no accumulation in thyroid was noted 48 hours later (1).

Groups of 6 rats of each sex were given 0, 2, 20, 200, 1000 or 2000 µg ^{14}C-ETU/day for 7 days. Doses were equivalent to 0, 0.1, 1, 10, 50 or 100 ppm in feed. The amount of ^{14}C in thyroid increased with increasing dose, but only up to 50 ppm. No further increase was noted at 100 ppm. Seventeen days after the last dose of ETU, the ^{14}C level in thyroids had declined by 80 to 94%. This shows that ETU and/or its metabolites do not accumulate permanently in the thyroid (57).

Most ETU is eliminated in urine. In an experiment in which two female rhesus monkeys were exposed to ^{14}C-ETU (40 mg/kg body weight, gavage) 47% and 64% of the radioactivity was recovered in urine within 48 hours. Feces contained less than 1.5% (1). In a similar experiment with rats and guinea pigs (20 mg/kg body weight) 65% (rats) and 47% (guinea pigs) of radioactivity was recovered in urine within 48 hours (61% and 45% within 24 hours) (64). In a 28-day study with rats it was found that the relative amounts of ETU in urine increased with increasing dose, possibly indicating that metabolism of ETU became saturated. At daily doses of 10.6, 17.6 and 23.4 mg/kg body weight, excretion in urine was on average 25%, 36% and 49% of the dose respectively (50).

For ETU and its metabolites, IPCS/WHO report a half time of 28 hours in monkeys (9.3 mg 2-^{14}C-ETU, per os), 9-10 hours in rats (240 mg/kg body weight, per os) and 5 hours in mice (240 mg/kg body weight, per os) (33, 71). The half time for ^{14}C-ETU (4 mg/kg body weight, i.v.) in the blood of 2 female cats was 3.5 hours (34). In humans, the half time for elimination of ETU via kidneys is

estimated to be between 32 and 100 hours (49, 52). It is possible that the long half time is due to slow uptake through the skin (52).

Biotransformation

Rats and cats were given oral doses of ^{14}C-ETU (4 mg/kg b.w.); 24-hour urine samples from the rats contained mostly unchanged ETU, ethyleneurea, 4-imidazoline-2-one and imidazoline, and those from the cats contained mostly S-methyl ETU, unchanged ETU and ethyleneurea (34). Biotransformation was more extensive in the cats than in the rats (34). Very small amounts of 1-methyl thio-urea were found in plasma of rats after oral administration of ETU (48). It was shown in a study with mice that biotransformation of ETU involves oxidation of the sulfur atom, with 2-imidazoline-2-yl-sulfenate as the primary product (78). There are no data on metabolic pathways in humans.

In mice, ETU is metabolized primarily by the microsomal flavin-containing monooxygenase system (FMO) (30). The FMO-dependent binding of ETU metabolites to proteins in the liver may contribute to the chronic liver toxicity that has been observed in mice (15, 30). Mice metabolize ETU more rapidly than rats do, which may explain why ETU shows acute toxicity but not teratogenicity in mice (see below). Oral administration of ETU (50 to 1000 mg/kg body weight) induced cytochrome P-450 (aniline hydroxylase: CYP2E1) activity in mice (61), but reduced the activity in rats (54, 61).

Nitrosation

N-nitroso-ethylenethiourea, a nitrosamide containing sulfur, may be formed from ETU in the presence of nitrite in acid environments. Nitrosamides spontaneously break down to carbonium ions at physiological pH, and are mutagenic without metabolic activation (47).

Sodium nitrite, which is used to preserve meat, is the primary dietary source of nitrites. In Europe, the daily intake of sodium nitrite is about 4 mg per person. Nitrates may also play some role, since they can be reduced to nitrites in the mouth. Intake of nitrate is mostly from vegetables, and on average amounts to about 100 mg per person per day. It can be assumed that about 6% of this (6 mg) is transformed to nitrite, increasing the daily nitrite intake to about 10 mg per person (81). The formation of *N*-nitroso-ETU is probably much less likely with inhalation or skin uptake of ETU than with oral exposure.

Biological monitoring

As mentioned previously, urine samples can be used for biological monitoring that reflects the past 24 hours of exposure to ETU. Analysis of ETU bound to hemo-globin has been proposed as a method for estimating longer exposures (up to 4 months). Of 15 workers occupationally exposed to mancozeb, 40% had identifiable Hb adducts of ETU (0.5-1.42 pmol/mg Hb) (69). It has been demonstrated in studies with rats that ETU, after metabolic activation –

presumably to a reactive sulfenic acid (see under Biotransformation) – forms covalent bonds to cysteine in hemoglobin in the form of a mixed disulfide. Since glutathione has the same ability to bind the reactive metabolite of ETU, only a very small proportion is bound to Hb. It appears that, at comparable exposures, more Hb adducts are formed in humans than in rats (69).

Toxic effects

Human data

In an English study from 1984, thyroid function was examined in eight production workers from a plant that produced ETU and five workers (mixers) from a factory where ETU was used in rubber production (84). Air concentrations of ETU ranged from 10 to 330 µg/m^3 in the production plant and from 120 to 160 µg/m^3 in the rubber factory. Thyroid function was measured as levels of T_4 (thyroxine), TSH (thyroid-stimulating hormone) and TBG (thyroxine-binding globulin) in serum. It was found that T_4 levels were lower in the mixers (geometric mean 80.5 nmol/l) than in the process workers (geometric mean 96.4 nmol/l) and an unexposed control group (geometric mean 105.7 nmol/l), but the individual values were within the range of normal reference values for T_4 (50 to 150 nmol/l) (53). TSH and TBG levels were normal in all the subjects except one mixer, who had an elevated TSH level (84).

The question of whether ETU is teratogenic was addressed in a study of 699 women of childbearing age who had come into contact with ETU at a rubber factory in Birmingham, England. Of these, 255 women were traced who had borne a total of 420 children. Only 59 of the women had worked at the factory during early pregnancy, and none of these had borne children with birth defects. In the entire group of 420 children there were 11 with birth defects; no more than predicted. Three of these children had been born before their mothers began work at the factory, and the other eight had been born at least a year after their mothers had quit working there (83).

There is a study on the incidence of thyroid cancer among 1,929 workers who had worked in several rubber factories and in a factory for production of ETU in England. No cases of thyroid cancer in this group had been reported to the regional cancer register between 1957 and 1971. The expected incidence of thyroid cancer was 2.6 per 100,000 (0.6 for men and 2.0 for women), which would be less than one case (0.05) in a group of this size (83).

An ecological study, not reviewed by referees (von Meyer WC, Philadelphia, PA: Rohm & Haas Company, 1977) is cited by Houeto *et al.* (29). In this study there was a trend (not statistically significant) to elevated incidences of liver and thyroid cancer in several parts of the United States where use of dithiocarbamate pesticides had increased.

A study of 49 Mexican workers who sprayed tomatoes with ethylenebisdithio-carbamate fungicides without using protective clothing or masks revealed elevated TSH levels (2.13 ± 0.15 mIU/liter; 1.61 ± 0.l9 mIU/liter in 24 unexposed

controls). Levels of T_4 were unaffected, however, and no symptoms of changes in thyroid function were observed, although no clinical examination was made. Exposure to ETU was estimated by measuring the concentration of ETU in morning urine the day after taking the blood samples used for the other analyses. The average level among the exposed subjects was 58 ± 26 ppb. All the controls and 34% of the exposed subjects had urine levels below the detection limit of 10 ppb. A cytogenetic examination revealed that the exposed workers had significantly elevated levels of sister chromatid exchanges and chromosome aberrations in the form of total translocations, but it is impossible to determine whether this damage was due to ETU or to other substances in the fungicides (85). Elevated frequencies of chromosome aberrations and sister chromatid exchanges are also reported in an earlier study of 44 workers exposed to mancozeb (36).

Patch tests with ETU (2% in vaseline) were given to 200 patients at a Polish dermatology clinic: there was one positive response (0.5%) (74). There is a reported case of allergic contact dermatitis in a 53-year-old woman who had worked in production of rubber goods for 13 years. She had a positive reaction to a patch test with ETU (1-0.01% in water). Results for 20 controls were negative (9). A positive reaction to ETU has also been reported in a dentist with contact eczema on the fingertips (37).

Among 11 cases of contact allergy after use of a rubber heat retainer, 6 of 7 tested patients had positive reactions to patch tests with ETU (1%), and all 7 of them had positive reactions to diphenylthiourea. This chemical could be identified in the heat retainers, and was probably the cause of the contact allergy. The role of ETU is less clear, since this substance could not be identified in the heat retainers (60).

Animal data: Short-term effects (up to 4 month

The acute toxicity of ETU is low. The reported LD_{50} for ETU given orally to rats is between 545 and 1830 mg/kg body weight. For oral doses to mice and hamsters, the LD_{50} is more than 3000 mg/kg body weight (58). Cats seem to be more sensitive (45). A lethal dose for skin exposure of pregnant rats (ETU dissolved in DMSO) has been reported to be about 2250 mg/kg body weight (86).

Skin

Ethylenethiourea apparently causes little skin irritation. The threshold value for an effect of ETU on the skin of guinea pigs was >10% in water (59). ETU was tested for allergenic potential in the guinea pig maximization test, and ranked as weak (59).

Thyroid

Repeated exposure to ETU inhibits thyroid function in laboratory animals (15). Rats (Wistar males) were given ETU in drinking water (0 to 300 mg/l, ad libitum) for 28 days. The treatment resulted in a dose-dependent (11-23 mg/kg body weight/day) inhibition in secretion of T_3 and T_4 and a tenfold increase of TSH. No

changes in thyroid were detected under an optical microscope, but electron microscopy showed some changes in thyroid follicular cells (51).

In a 13-week study, F344/N rats (10 of each sex per group) were given feed containing 60, 125, 250, 500 or 750 ppm ETU (66). Histopathological changes were seen in thyroid and pituitary of both males and females. Diffuse hyperplasia in thyroid follicular cells was observed in both sexes at all dose levels. The NOAEL was therefore reported to be below 60 ppm in feed (≈ 3.0 mg/kg body weight/day for males and 4.3 mg/kg body weight/day for females) (66).

In a 90-day study, Sprague-Dawley rats (both sexes, 12 per group) were exposed to 75 or 100 ppm ETU in feed. At 100 ppm the serum level of T_4 was reduced and the T_3/T_4 quotient and TSH levels were elevated in the males, while there was a smaller effect on the females. At 75 ppm the T_4 levels were reduced in both sexes, but since neither T_3, TSH nor thyroid weights were affected, the animals were regarded as having normal thyroid function (67).

In another 90-day study with rats, it was found that 125 mg ETU/kg feed (125 ppm) reduced levels of T_3 and T_4 and markedly raised levels of TSH, and also enlarged thyroids, whereas 25 ppm yielded lower levels of T_4 and thyroid hyperplasia on day 60 – which, however, was not seen on either day 30 or day 90. The authors gave a NOAEL of 25 ppm (≈ 1.8-2.2 mg/kg body weight/day) for 90 days of exposure to ETU in feed (22). The Dutch criteria group made a different assessment, and gave a NOAEL of 5 ppm (≈ 0.4 mg ETU/kg body weight/day) (15).

Groups of 10 Osborne-Mendel rats (males) were given feed containing 0, 50, 100, 500 or 750 ppm ETU for up to 120 days (25). Relative thyroid weights were elevated at all examination times (30, 60, 90 and 120 days) in the animals receiving at least 100 ppm ETU in feed, but only at the last examination in the rats receiving 50 ppm. Thyroid weights were slightly but significantly elevated at the two lowest doses (at most 133% of controls), but thyroid weights in animals exposed to 500 or 750 ppm were about 5 times those of controls. An effect on thyroid function, measured as reduced uptake of ^{131}I, was observed only in the two highest dose groups after 4 hours, and also in the 100-ppm dose group after 24 hours. No histological changes were observed in the thyroids of rats given 50 ppm ETU in feed (25). In the assessments of the IARC (31) and DECOS (15), 50 ppm ETU in feed (according to DECOS, about 3.7 mg/kg body weight/day) should be regarded as the NOAEL in this study.

Young Wistar rats (males, 80-90 g) were exposed to ETU in feed for 5 days. A slight but significant elevation of TSH and reduction in levels of free T_4 were observed at 5 ppb, but not 500 ppb (63). The authors suggest that the reversed dose-response relationship might be due to tolerance development or more rapid detoxification.

An unpublished report (reviewed in Reference 3) describes an inhalation study (nose-only exposure) with Wistar rats, in which groups of 5 males and 5 females were exposed to 0, 10, 40, or 200 mg/m^3 ETU 6 hours/day, 5 days/week for 4 weeks. The particle size suggests that the ETU penetrated deep into the lungs. The

animals in the two highest dose groups had lower body weights and lower feed intake. The number of reticulocytes in the highest dose group (200 mg/m^3) was half that of controls. Effects on thyroid – lower T_4, histological changes – were dose-dependent, and observed at 40 mg/m^3 and above. Hyperplasias in the anterior pituitary and in mandibular glands were also observed. The NOEL was reported to be between 10 and 40 mg/m^3.

A series of biochemical experiments made to elucidate the mechanism behind ETU's effect on thyroid showed that ETU inhibits thyroid peroxidase. The inhibition occurred only in the presence of iodide, and involved simultaneous oxidation of ETU to imidazoline and bisulfite. Inhibition of thyroid peroxidase ceased when all the ETU had been oxidized. ETU did not form covalent bonds to thyroid peroxidase. Since the inhibition was reversible, occasional exposure to small amounts of ETU should not have much effect on thyroid function (16).
In summary, several short-term studies of thyroid effects have shown that the NOAEL for rats is in the range 0.4 to 4 mg/kg body weight/day. For mice, which are less sensitive than rats, the NOAEL for thyroid effects is 50 mg/kg body weight/day (15).

Liver
Effects on liver (increased liver weight, triglycerides in liver, fatty degeneration) were observed in rats 24 hours after doses of 920 mg ETU/kg body weight (gavage) (90). DECOS (15) reports a study of male rats that received ETU in drinking water for up to 8 months. Liver morphology was not affected by 50 mg/l (15 mg/kg body weight/day) whereas 500 mg/l had effects which included increasing the amount of smooth endoplasmic reticulum.

Nervous system
Effects on the peripheral nervous system were observed in rats given 600 ppm ETU in feed for 4 weeks (90). Toxic effects on the central nervous system were observed in 4 of 7 pregnant cats given 10 mg ETU/kg body weight/day for 20 days (45). The results of a study in which ETU was given to rats in drinking water (0 to 300 mg/l) led the authors to state that the target organ for ETU's neurotoxic effect was cholinergic peripheral nerves rather than the CNS (77).

Kidneys
In a 28-day study, rats were given drinking water containing 0, 100, 200 or 300 mg/l ETU (≈ 0, 11, 18, or 23 mg ETU/kg body weight/day) and effects on kidneys were examined (50). Weight gain in the two highest dose groups was lower than in controls, possibly because of slight dehydration, since these animals drank less than normal. No significant changes in urine composition were found (Na, K, protein, glucose, uric acid, specific gravity, vasopressin). Examination under optical microscope revealed no histological changes in the kidneys, but electron microscopy revealed changes in the proximal tubuli of animals exposed to 300 mg/l. In another study with rats, in which ETU was given by gavage in single doses of 50 to 500 mg/kg body weight, dose-dependent indications of

kidney damage (including proteinuria) were observed at doses of 100 mg/kg or higher (55).

Animal data: long-term effects

Mice and rats

The NTP made a long-term exposure study in which $B_6C_3F_1$ mice and F-344/N rats were given ETU in feed (66). The study combined perinatal exposure with a conventional NTP protocol for studies of chronic toxicity. Long-term exposure of the mice caused non-neoplastic damage to thyroid, liver and pituitary (11, 66) Vacuolization of cytoplasm in thyroid follicular cells was observed in both male and female mice exposed to 330 ppm ETU (≈ 66 mg/kg b.w./day) for 2 years (LOAEL). Levels of T_4 were significantly lower in both sexes, and TSH levels were slightly elevated (11).

The ETU-exposed rats showed thyroid damage but no non-neoplastic damage to liver or pituitary (11, 66). Thyroid hyperplasia was observed in both male and female rats exposed to 83 ppm for 9 months, and was accompanied by significant reductions of T_3 and T_4 and elevated TSH. A lower concentration (25 ppm) also had effects on T_3 , T_4 and TSH in the animals that had been exposed perinatally to 9 ppm. At the close of the two-year study no histopathological effects were observed in the rats exposed to levels below 83 ppm, but 60 to 90% of those exposed to 83 ppm and 250 ppm had hyperplasias in thyroid follicular cells (11). In a French study, groups of 20 male and 20 female rats were exposed to 0, 5, 17, 60 or 200 ppm ETU in feed for two years (23). Reduced food intake and effects on body weight were reported at 17 ppm and higher. Significantly elevated serum cholesterol levels were found in all dose groups and both sexes. The elevations were constant over time (3 to 24 months) and dose-dependent: 5 ppm ETU raised the cholesterol level by about 30%, and 200 ppm by about 80%. Slightly elevated serum levels of the hepatic enzymes alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were also observed, but they were temporary and not clearly related to the dose of ETU. The intake of ETU at 5 ppm in feed was calculated to be about 0.37 mg/kg b.w./day at one month of age, and 0.22 to 0.26 mg/kg b.w./day at 3 months and older.

Hamsters

In conjunction with the study described above, 20 hamsters of each sex per group were exposed to ETU in feed for 20 months. Dose levels were 0, 5, 17, 60 or 200 ppm (23). Reduced food intake and lower body weights were observed at 60 ppm and higher. As with the rats, cholesterol levels in serum in both sexes and at all dose levels and all times were significantly above those of controls. At the end of the 20-month exposure, ALP and ALT levels were also significantly elevated (about 40% at most) in both sexes at all dose levels. Glucose-6-phosphate dehydrogenase in the liver was significantly lower (as much as 60%) in both sexes at all dose levels.

Dogs

Beagles of both sexes have also been experimentally exposed to ETU. Exposures have been for 4, 13, or 52 weeks. These studies have not been published, but have been assessed by the FAO/WHO expert panel (20). In the 4-week study, the dogs (2 of each sex per group) were exposed to concentrations of 0, 200, 980 and 4900 ppm in feed. Reduced weight gain, lower T_4 and T_3 levels and thyroid enlargement were observed at 980 ppm. In the 13-week study, the dogs (4 of each sex per group) were exposed to 0, 10, 150 and 2000 ppm in feed. No effects were noted at 10 ppm (NOAEL), which according to the WHO expert group is equivalent to 0.39 mg/kg b.w./day. At 150 and 2000 ppm there were statistically significant reductions of hemoglobin, hematocrit and red blood cells, and a statistically significant elevation of cholesterol level. Effects on the thyroid were seen only at 2000 ppm. In the 52-week study, the dogs (4 of each sex per group) were exposed to 0, 5, 50 or 500 ppm ETU in feed. No effects were observed at 5 ppm (NOAEL). The 50 ppm exposure resulted in reduced weight gain, thyroid hypertrophy with colloid accumulation, slight increase in thyroid weight and an accumulation of pigment in the liver (20).

Monkeys

Rhesus monkeys caught in the wild (5 of each sex per group) were exposed to ETU in diet for about 6 months in two studies which have not been published but are mentioned by the FAO/WHO expert panel (20). The studies report increased uptake of ^{125}I at a concentration of 50 ppm, and elevated thyroid and spleen weights in males at 150 ppm and above and in females at 50 ppm and above. These studies were judged to be unreliable, however, since the monkeys were not entirely healthy (20).

Genotoxicity, mutagenicity

The results of various short-term tests published prior to 1993 have been summarized and evaluated by Dearfield (14). The overall impression from the large number of bacterial tests made with ETU is that the substance has weak but dose-dependent mutagenic activity which is not apparent at concentrations below 1000 µg per plate (20 ml medium), and that the mutations are base-substitutions. High concentrations have caused aneuploidy (incomplete chromosome separation) in yeast cells, mutations in *Tradescantia* plants, and gene mutations and chromosome aberrations in mammalian cells. *In vivo* tests with mammals have usually been negative (14).

Subsequently published studies report aneuploidy in yeast at a concentration of about 500 µg/l, and inhibited mitosis and elevated numbers of chromosome aberrations in onions at concentrations of 2.5 and 25 µg/ml (21). Increased numbers of somatic mutations were observed in two insecticide-resistant strains of *Drosophila* when the larvae were raised on food containing 50 or 100 mg ETU/liter (70). The Comet assay was used to identify and quantify the DNA damage (alkaline labile sites) in mice that had been treated with ETU (76). ETU

was tested along with 7 other substances that cause hepatic cancers in experimental animals but have not been shown to cause micronuclei in the bone marrow cells of mice. The mice were killed 3 hours and 24 hours after receiving a single intraperitoneal dose of 2000 mg/kg body weight. The ETU caused DNA damage in cells from liver, lung, spleen, kidney and bone marrow.

Mutagenicity in bacteria is greatly increased if ETU is combined with nitrite, and *N*-nitroso ETU is strongly mutagenic in bacterial tests (79, 80, 82). A remarkable sensitivity to ETU plus sodium nitrite was observed in two studies using the host-mediated assay method (8, 82). In mice given an oral dose of 50 mg $NaNO_2$/kg body weight, there was a significant, dose-dependent increase in the number of mutations in *Salmonella typhimurium* G46 when the mice were simultaneously given ETU in doses of 1 to 25 mg/kg body weight (82).

The interaction between ETU and sodium nitrite has also been studied with regard to induction of dominant-lethal mutations in mice (88). Daily doses of ETU (150 mg/kg b.w.) and $NaNO_2$ (50 mg/kg b.w.) were given orally for five consecutive days to male mice, which were mated with groups of untreated females for the following six weeks. The females mated six weeks after the treatment had a greatly reduced proportion of pregnancies as well as lower numbers of implants and living embryos. The delayed effects were regarded as an indication that ETU in the presence of $NaNO_2$ forms *N*-nitroso ETU and damages the stem cells (spermatogonia). An increase in the number of genetic aberrations was also seen in stem cells of mice after treatment with 100 mg *N*-nitroso ETU/kg b.w. Neither ETU nor *N*-nitroso ETU has been tested on mammals with methods that can reveal hereditary (non-lethal) changes (e.g. specific locus test, mouse spot test).

In brief, ETU is regarded as weakly genotoxic because of the dose-dependent increases of gene mutations observed in bacteria and the results of a few tests with yeast cells, plants, fruit flies, mammalian cells and laboratory mammals (*in vivo*), which have shown genotoxic effects at high exposure levels. Most *in vivo* tests with mammals have been negative, however. *N*-nitroso ETU, on the other hand, is a powerful genotoxin both *in vitro* and *in vivo*. Endogenous formation of *N*-nitroso ETU, which occurs mostly in acidic environments, must be considered when assessing both the genotoxicity of ETU and its potential carcinogenic effect. The probability of *N*-nitroso ETU formation with ETU exposure must be much lower with inhalation or skin uptake than it is with oral exposure.

Earlier assessments of genotoxicity

In 1987, the IARC summarized data from genotoxicity tests in the form of a genotoxicity profile (32). Positive results were reported only from tests with prokaryotes and lower eukaryotes. ETU was classified as non-genotoxic in an assessment of pesticides made for a FAO/WHO program (20). The NTP reported that ETU had been thoroughly tested for genotoxicity using numerous test methods both *in vivo* and *in vitro*, and with few exceptions results had been negative (66). DECOS, which set a health-based exposure limit for ETU (15), reported that ETU *per se* is non-mutagenic. A 1995 review article described ETU

as non-genotoxic in mammalian systems and proposed that ETU causes liver tumors in mice by a non-genotoxic mechanism (18). Dearfield (at the EPA) made the overall assessment that ETU could not be regarded as lacking genotoxic activity (14). The genotoxic effect was judged to be weak, but it was pointed out that nitrosation creates a mutagenic product that may be more potent.

Carcinogenicity

The results of cancer tests are summarized in Appendix 1. Most of these studies were made with rats. Both short-term and long-term toxicity studies have shown that species differ in both sensitivity to ETU and the organs affected.

Mice

Elevated incidences of hepatic adenomas and carcinomas have been reported in mice at a dose of 66 mg/kg/day (330 ppm ETU in feed) (11, 66). Male and female mice were exposed perinatally from minus one up to eight weeks of age (F_0) and/or as adults (F_1) to between 0 and 1000 ppm ETU in feed. The mice exposed to 330 ppm perinatally only showed no tumors after two years. Those exposed to 330 ppm as adults only had tumors in liver, pituitary or thyroid. For adult animals exposed to 330 ppm, the incidences of thyroid and pituitary tumors were marginally higher in animals that had also been exposed perinatally. Perinatal exposure to 300 ppm, however, had no effect on tumor incidence in mice exposed to the highest dose (1000 ppm) when they were compared to the group not exposed perinatally.

Yoshida *et al.* (93) made a study in which ETU was given to mice (Crj:CD1) in combination with sodium nitrite. The mice were given ETU and sodium nitrite (in water) by gavage once a week for ten weeks, in the following combinations (ETU + $NaNO_2$): 0 + 0; 100 + 0; 0 + 70; 25 + 17.5; 50 + 35 and 100 + 70 mg/kg body weight. The animals were observed for 18 months beginning with the first treatment. It was found that ETU combined with sodium nitrite caused an earlier appearance of tumors and/or a dose-dependent increase of tumors in lymphatic tissue, lung, stomach, Harder's gland and uterus. The tumor locations were thus not the same as those observed after administration of ETU alone (see 11, 66). No carcinogenic effect was observed in mice given ETU or sodium nitrite alone. A dose-dependent increase of pulmonary adenomas and adenocarcinomas was observed in both females and males, and the number of females with pulmonary adenomas or adenocarcinomas was significantly elevated in the group given (ETU + $NaNO_2$) 25 + 17.5 mg/kg b.w./week. These results suggest that ETU is transformed *in vivo* to *N*-nitroso ETU and that *N*-nitroso ETU has a stronger carcinogenic effect on mice than ETU alone. This has been confirmed in a study of tumor induction in mice (ICR females), in which oral administration of *N*-nitroso ETU in doses of 0.66 to 2.64 mg (26.4 to 105.6 mg/kg b.w.) once a week for ten weeks increased the incidence of pulmonary tumors and lymphocytic neoplasms (62).

Rats

For rats, the thyroid has been found to be the organ most sensitive to both short-term and long-term exposures. Dose-dependent increases of thyroid tumors have been observed in a number of different studies with rats (11, 23, 24, 26, 91, 92). Graham *et al.* reported increased appearance of thyroid tumors at 250 ppm or more in feed (24, 26), but not at 125 ppm. Calculating from information given by the authors, exposure to 125 ppm in feed is equivalent to about 10 mg/kg body weight/day (NOAEL). Gak *et al.* (23) reported no thyroid tumors after 20 months of exposure to 17 ppm in feed (according to the authors, equivalent to 1.27 mg/kg b.w./day or less).

In the NTP study, male and female rats were exposed perinatally from minus one up to eight weeks of age (F_0) and as adults (F_1) to 0-250 ppm ETU in feed. There was a clear increase of hyperplasia in thyroid follicular cells after 2 years of exposure to 83 or 250 ppm. Thyroid follicular cell adenomas or carcinomas were seen in about 20% of animals exposed to 83 ppm and in about 60% of those exposed to 250 ppm. Male rats were more sensitive than females to the carcinogenic effects of ETU. In addition to the dose-dependent increases of thyroid tumors, there was also a small but significant increase of tumors in Zymbal's glands (both sexes at F_0 90 ppm, F_1 250 ppm) and mononuclear cell leukemia (both sexes at F_0 90 ppm, F_1 250 ppm; males at F_0 90 ppm, F_1 83 ppm) (11). The LOEL was 83 ppm, which according to DECOS (15) is equivalent to 6.23 mg/kg b.w./day.

The ability of thioureas (e.g. ETU and thiourea) to cause thyroid tumors is attributed to hormonal disturbances. Rats are regarded as a sensitive species in this respect. Thiourea inhibits the enzyme thyroid peroxidase, which causes serum levels of thyroid hormones T_3 and T_4 to decline. This in turn stimulates the hypothalamus and pituitary, and more thyroid-stimulating hormone (TSH) is produced. TSH stimulates thyroid growth, and chronically elevated levels of TSH in serum can cause thyroid hyperplasia which may eventually develop into tumors (2, 27, 28).

When female rats were given simultaneous doses of ETU (80 mg/kg body weight) and $NaNO_2$ (56 mg/kg body weight) once per week from 11 to 51 weeks of age, 13% of animals developed adenocarcinomas in uterine endometrium (65). No such tumors were observed in controls.

Teratogenicity

ETU is strongly teratogenic to rats (10, 12, 41, 56, 72, 75). It can also have embryotoxic effects on mice (10, 39), rabbits (41), cats (45), hamsters (10, 46), and guinea pigs (10). ETU causes elevated mortality and a low incidence of malformations in some of these species, but only at high dose levels (12, 40). In an aquatic *in vitro* assay for embryotoxic effects on water fleas (*Daphnia magna*), a significant increase in the incidence of malformations was seen at an ETU concentration of 20 mg/liter (68).

The lowest single oral dose that yields developmental anomalies in rats (LOAEL) is 40 mg/kg body weight. For repeated doses, the lowest exposure is 10 to 20 mg/kg/day on days 6 to 15 of gestation (41, 72). Maternal toxicity was observed at 80 mg/kg/day, and somewhat retarded ossification was observed in a third of fetuses after repeated administration of 5 mg/kg (41). Brain damage is the most common teratogenic effect in rats. ETU causes craniocele, meningo-encephalocele, hydrocephalus, obliteration of the neural canal and enlarged brain ventricles. Skeletal damage is also common, and includes club foot, short and crooked tails, and rib anomalies. ETU shows different types of teratogenic effects, determined by the stage of gestation at which the mother was exposed. It was shown in one study (72) that effects on the eyes appeared only after treatment on days 10 and 11, tail defects after treatment on days 11– 14, and cleft palate after treatment on days 12-16. Damage to toes on forepaws appeared at earlier exposures than damage to toes on hindpaws. In a study of teratogenic effects of ETU in thyroidectomized females and controls given false thyroidectomies, it was concluded that ETU-dependent changes in thyroid function or thyroxine levels in the mothers was probably not the explanation for the teratogenic effects of ETU (56).

Rat embryos examined after *in vitro* exposure to ETU show damage, primarily to the tail and head, at concentrations of 10 mg/liter or higher (13, 35, 42, 89). Both early (days 10-13) and late (day 19) prenatal exposure damages nervous tissue (13, 40). Neural cells have been identified as particularly sensitive to the toxic effects of ETU, both by examination of cells and tissues from exposed embryos and by exposure of cultured embryonic cells (13, 40, 89).

For mice, the lowest single dose with embryotoxic effect (LOAEL) is 1600 mg/kg (39); for repeated exposures, the lowest dose level is more than 200 mg/kg (10). Rats are thus twenty to forty times more sensitive than mice, although the teratogenic effects of ETU are of the same types in both species (13). The difference in metabolic capacity between rats and mice leads to higher blood levels of ETU in rats than in mice (see Biotransformation) (73). The effect of maternal metabolism was examined by adding S9 from Aroclor-1254 induced rat or mouse liver together with a NADPH-generating system to embryos exposed to ETU *in vitro* (13). S9 from mouse completely neutralized the teratogenic effect of ETU on both rat and mouse embryos. The differences in metabolism may explain some of the differences in sensitivity between rats and mice, but rat embryos and cultured brain cells from rats are also more sensitive to the toxic effects of ETU than similar tissues from mice with exposure *in vitro* (13, 89).

Effects of nitrosation

In the presence of $NaNO_2$ ETU is nitrosated to a substance that is teratogenic to mice (87): 400 mg/kg ETU given together with 200 mg/kg $NaNO_2$ is embryotoxic and teratogenic, causing primarily skeletal anomalies when administered on day 6, 8, or 10, but not when administered on day 12 of gestation. The $NaNO_2$ has effect only when given within about an hour of the ETU exposure (87). The reported

damage includes deformed tails and ribs, omphalocele, cleft palate, high frequency of deformed vertebra, fused lung lobes, missing kidneys (kidney agenesis), small or missing eyes and swollen brain ventricles (87).

N-nitroso ETU causes hydrocephalus in rat embryos (44). It has been observed, however, that $NaNO_2$ almost completely neutralizes the teratogenic effects of ETU in rats treated on day 13 or 15 of gestation (43).

Dose-effect/dose-response relationships

It was found in one study that occupational exposure to ETU at air concentrations in the range 120–160 mg/m^3 inhibits thyroid function, measured as somewhat lower levels of the thyroid hormone T_4 (thyroxine). Levels of T_4 were lower in exposed workers (geometric mean 80.5 nmol/l) than in unexposed controls (geometric mean 105.7 nmol/l), but the individual values were all within normal reference limits for T_4 (84). Assuming an air intake of 10 m^3/day and a body weight of 70 kg, exposure to an air concentration of 120 µg/m^3 would result in uptake of about 17 µg/kg/day. Skin uptake is likely, and may be high.

Elevated TSH levels were observed in Mexican farm workers exposed to fungicides containing ETU. ETU levels in urine were on average 58 ppb (µg/liter) (85). Assuming a urine volume of 2 liters, that half of absorbed ETU is excreted in urine, and that uptake is complete, this is equivalent to a single dose of 3 to 4 µg/kg.

Considering the widespread use of ETU, there are few reported cases of contact allergy.

Relevant information from animal experiments with oral exposure is summarized in Table 1. In the only (unpublished) inhalation study with rats, effects on thyroid were noted at exposure to 40 mg/m^3 ETU 6 hours/day (3). If it is assumed that air intake is 0.2 m^3/day and body weight is 0.33 kg, this exposure is equivalent to 6 mg/kg/day. Exposure to 10 mg/m^3 had no effect.

Conclusions

Data from occupational exposures indicate that the critical effect of occupational exposure to ETU is its effect on the thyroid. This effect has also been observed in experimental animals. ETU is carcinogenic to experimental animals. Simultaneous exposure to ETU and nitrite in food yields tumors at lower ETU levels and in other organs. ETU is considered to be slightly genotoxic, whereas *N*-nitroso ETU is strongly genotoxic. ETU is teratogenic to experimental animals. The teratogenic effect of ETU in different species seems to be inversely related to the biotransformation rate. There are neither qualitative nor quantitative data on biotransformation in humans. A few cases of contact allergy have been reported after skin contact with ETU, but the allergenic potential of ETU is probably low. Animal studies suggest that skin uptake may be high.

Table 1. Effects observed in experimental animals given ETU in feed.

Dose (mg/kg/day)	Concentration in feed (ppm)	Duration of exposure	Level value for effect, species, effect	Ref.
0.2 - 0.4	5	24 months	LOEL, rat elevated serum cholesterol	23
0.4 - 0.7	5	20 months	LOEL, hamster elevated serum cholesterol	23
ca. 2	25	60 days	LOEL, rat effects on thyroid	22
ca. 2	25	90 days	NOEL, rat effects on thyroid	22
ca. 2	50	52 weeks	LOEL, dog effects on thyroid	20
3.7	50	120 days	NOAEL, rat effects on thyroid	25
3 - 4.3	60	13 weeks	LOAEL, rat thyroid hyperplasia	66
5		until day 15 of gestation	LOAEL, rat retarded ossification	41
6.2	83	24 months	LOAEL, rat thyroid cancer, effects on thyroid	11
10		until day 15 of gestation	LOAEL, rat teratogenic effects	41
10		20 days	LOAEL, cat CNS toxicity	45
23		28 days	LOEL, rat structural changes in renal tubules	50
66	330	24 months	lowest tested dose, mouse liver cancer, effects on thyroid	11

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Appendix 1. Results from carcinogenicity tests with experimental animals exposed to ethylenethiourea (ETU).

Species	Method of administration, dose		Exposure time	Effect (comments)	Ref.
Mouse ($B_6C_3F_1$) both sexes 50 per group	in diet	330 ppm	perinatal period (-1 through +8 weeks)	No increase in tumor incidence	11, 66
		33 ppm +100 ppm	perinatal period 24 months	No increase in tumor incidence	
		330 ppm +1000 ppm	perinatal period 24 months	Elevated numbers of adenomas and carcinomas in liver, thyroid and pituitary	
		330 ppm (66 mg/kg bw/day)	24 months	Males: 32/50 with liver tumors females: 44/50 with liver tumors	
		1000 ppm (200 mg/kg bw/day	24 months	Elevated numbers of adenomas and carcinomas in liver, thyroid and pituitary	
Mouse (Crj;CD-1) both sexes 60 per group	gavage	100 mg/kg bw once a week	10 weeks observed 18 months	No increase in tumor incidence (25 mg/kg/week ETU + 17.5 mg/kg/week $NaNO_2$ increased the incidence of pulmonary tumors in females)	93
Rat (Charles River) both sexes 69-73 per group	in diet	5 ppm (0.4 mg/kg bw/day)	24 months	2/75 with thyroid tumors (2/72 controls with thyroid tumors)	24, 26
		25 ppm (2 mg/kg bw/day)	24 months	1/73 with thyroid tumor	
		125 ppm (10 mg/kg bw/day)	24 months	2/73 with thyroid tumor (metastases observed in lungs)	

Appendix 1. Cont.

Species	Method of administration, dose		Exposure time	Effect (comments)	Ref.
		250 ppm (20 mg/kg bw/day)	24 months	16/69 with thyroid tumors	
		500 ppm (40 mg/kg bw/day)	24 months	2/75 with thyroid tumors	
Rat (F344/N) both sexes 50 per group	in diet	0 +0	perinatal period 24 months	Males: 1/49 with thyroid tumors females: 3/50 with thyroid tumors	11, 66
		90 ppm	perinatal period (-1 to +8 weeks)	No increase in tumor incidence	
		30-90 ppm +83-250 ppm	perinatal period 24 months	Higher incidence of follicular adenomas and carcinomas in thyroids	
		83 ppm (6.23 mg/kg bw/day)	24 months	Males: 12/46 with thyroid tumors females: 7/44 with thyroid tumors	
		250 ppm (18.75 mg/kg bw/day)	24 months	Males: 37/50 with thyroid tumors females: 30/49 with thyroid tumors	
Rat (Charles River) both sexes 26 per group	in diet	175 ppm (13 mg/kg bw/day)	18 months	6/26 with thyroid tumors (no tumors in controls)	91, 92
		350 ppm (26 mg/kg bw/day)	18 months	Males: 17/26 with thyroid tumors females: 8/26 with thyroid tumors (observation period 6 months)	

Appendix 1. Cont.

Species	Method of administration, dose		Exposure time	Effect (comments)	Ref.
Rats both sexes 20 per group	in diet	5 ppm (0.2-0.4 mg/kg bw/day)	20 months	No increase in tumor incidence	23
		17 ppm (0.7-1.3 mg/kg bw/day)	20 months	Slight (not statistically significant) increase of malignant tumors in thyroids	
		60 ppm (2.5-4.7 mg/kg bw/day)	20 months	Significant increase of malignant tumors in thyroids	
		200 ppm (8-16 mg/kg bw/day)	20 months	Significant increase of malignant tumors in thyroids	
Hamsters both sexes 20 per group	in diet	5 ppm (0.4-0.7 mg/kg bw/day)	24 months	No increase in tumor incidence	23
		17 ppm (1.3-2.6 mg/kg bw/day)	24 months	No increase in tumor incidence	
		60 ppm (3.8-8.5 mg/kg bw/day)	24 months	No increase in tumor incidence	
		200 ppm (11-26 mg/kg bw/day)	24 months	No increase in tumor incidence	

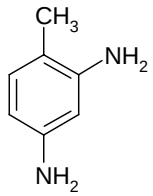
Consensus Report for Toluene-2,4-diamine and Toluene-2,6-diamine

November 1, 2000

Physical and chemical data. Uses

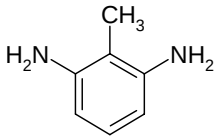
toluene-2,4-diamine (2,4-TDA)

CAS No.:	95-80-7
Synonyms:	2,4-diaminotoluene
	1,3-diamino-4-methylbenzene
	5-amino-o-toluidine
	4-methyl-1,3-benzene diamine
	2,4-toluenediamine
Formula:	$C_7H_{10}N_2$
Structure:	



Molecular weight:	122.17
Melting point:	99 – 100 °C
Boiling point:	285 – 292 °C
Density:	1.042 g/ml
Flash point:	169 °C
Conversion factors:	1 ppm = 5.07 mg/m^3 (20 °C)
	1 mg/m^3 = 0.19 ppm (20 °C)

toluene-2,6-diamine (2,6-TDA)

CAS No.:	823-40-5
Synonyms:	2,6-diaminotoluene 1,3-diamino-2-methylbenzene 2-methyl-1,3-benzenediamine 2,6-toluenediamine
Formula:	$C_7H_{10}N_2$
Structure:	

Molecular weight:	122.17
Melting point:	105 – 106 °C
Density:	1.031 g/ml
Flash point:	125 °C
Conversion factors:	1 ppm = 5.07 mg/m^3 (20 °C) 1 mg/m^3 = 0.19 ppm (20 °C)

At room temperature toluene-2,4-diamine (2,4-TDA) and toluene-2,6-diamine (2,6-TDA) are clear crystals. Both substances are soluble in water (2,4-TDA: 37.8 g/l at 20 °C; 2,6-TDA: 60 g/l at 15 °C), alcohol, ether, and many polar solvents.

Both 2,4-TDA and 2,6-TDA are used primarily as intermediates in production of diisocyanates and other chemical substances. They are also used in production of urethane materials, paints and corrosion inhibitors. Some developing fluids contain TDA. Persons exposed to 2,4- or 2,6-toluene diisocyanate have been found to have 2,4-TDA or 2,6-TDA, respectively, in hydrolyzed urine (31). World production of 2,4-TDA and 2,6-TDA in 1993 was reported to be about 650,000 metric tons (15).

Uptake, biotransformation, excretion

2,4-TDA can be absorbed via the skin and digestive tract. Skin uptake by humans has been measured using ^{14}C-2,4-TDA: 4 µg 2,4-TDA dissolved in acetone was applied to the arm, and 24% of the dose was absorbed within 24 hours (29). Excretion in urine was highest after 4 to 8 hours. Skin absorption varies considerably, depending on the solvent used (21). It has been demonstrated in studies with rats that 2,4-TDA (3 or 60 mg/kg) is absorbed in the digestive tract and rapidly distributed to body tissues (42).

Rats were given ^{14}C-2,4-TDA intraperitoneally (77 mg/kg i.p.), and 4 to 24 hours after the injection the highest concentrations of radioactivity were found in liver and kidneys (18, 20). Mice were injected with the same substance (0.66

mg/kg i.p.), and 30 minutes later the highest tissue concentrations of radioactivity were in kidney, testis, epididymis and lung; after 1 hour the concentration was highest in liver (12% of the dose) (43).

In general, it appears that in most species only a small portion (0.1 to 3%) of 2,4-TDA is excreted unchanged, and the rest is metabolized (42, 44). The first step is hydroxylation, followed by N-acetylation. Both mono- and diacetyl derivatives have been identified in urine (3, 42, 44). The primary metabolite in urine is 5-hydroxy-2,4-TDA, but mice and rats differ in the occurrence and relative quantities of the various metabolites and conjugates (43). *In vitro* studies have shown that N-acetylation occurs mostly in the liver (17). The reactive metabolites are formed by P-450-dependent N-hydroxylation and sulfation (2, 12, 13, 20). It has been demonstrated in several studies that 2,4-TDA can form adducts with DNA and hemoglobin (4, 11, 25, 27).

2,4-TDA is excreted rapidly, most of it in urine. In rodents, elimination is biphasic: rapid elimination for 7 hours is followed by a slower phase (18, 43). In a study with rats it was determined that in the slower phase the half time for elimination in urine was 4.6 hours after intravenous administration (3 mg/kg) and 8 hours after oral administration (3 mg/kg) (42). With intraperitoneal administration (77 mg/kg, rats), 69% of the dose was recovered in urine and feces within 24 hours (18). Chronic exposure has been found to increase elimination in urine and decrease excretion in feces (43). 2,6-TDA can form hemoglobin adducts (34, 47, 49).

2,6-TDA is rapidly absorbed in the digestive tract. It undergoes hydroxylation and N-acetylation, and the primary metabolites found in urine are 2,6-diacetyl-aminotoluene and 6-acetylamino-2-amino-3-hydroxytoluene (8). Rats given 10-milligram oral doses of ^{14}C-2,6-TDA eliminated 85% of the radioactivity in urine within 24 hours. No unmetabolized 2,6-TDA was found in the urine. After 6 days, 1% of the radioactivity could still be detected in tissues (7).

Both 2,4-TDA and 2,6-TDA have been identified in hydrolyzed urine of persons exposed to toluene diisocyanates (TDI), and there are some studies of excretion of 2,4-TDA and 2,6-TDA by persons exposed to TDI (31). In one study of TDI-exposed workers, the half time in urine was determined to be 18 days for 2,4-TDA and 19 days for 2,6-TDA, and the half time in plasma 7.8 days for 2,4-TDA and 9.6 days for 2,6-TDA (28). In another study, in which volunteers were exposed to TDI for 4 hours, the half time in plasma for 2,4-TDA and 2,6-TDA was 2 to 5 hours during the initial elimination phase and more than 6 days in the slower phase (5).

Toxic effects

Human data

There is no published information regarding effects on human health resulting from exposure to 2,4-TDA or 2,6-TDA.

Animal data

2,4-TDA

One published study reports that 2,4-TDA causes very little or no skin irritation (14).

The reported LD_{50} values for 2,4-TDA range from 73 to 500 mg/kg body weight, depending on species and method of administration. The reported LC_{50} values for inhalation exposure are 120 – 150 mg/m^3 for mice and 916 mg/m^3 for rats (15). High doses of 2,4-TDA cause methemoglobin formation in several species (37, 45). Cats were found to be the most sensitive species: a dose of 10 mg/kg (i.p.) resulted in elevated numbers of Heinz inclusion bodies (37), regarded as an indication of oxidative stress.

Mice were given 2,4-TDA in oral doses of 25, 50 or 100 mg/kg/day for 14 days. The treatment resulted in changes in hepatic enzymes in serum, and the highest dose resulted in higher liver weights. Immunotoxic effects – higher numbers of B cells in spleens and lower spleen weights – were observed at all exposures. The immunotoxic effects were attributed to disruptions in differentiation and maturation of leukocytes (6, 46).

In a 7-week study, rats and mice were given 2,4-TDA in diet. The rats given feed containing 1000 ppm 2,4-TDA (≈75 mg/kg body weight/day) had lower body weights, elevated hematopoiesis and liver changes. The same diet given to mice resulted in reduced body weights but no histopathological changes. The NOEL for the 7-week exposure was reported to be 250 ppm for rats and 200 ppm for mice (32). In a two-year NTP cancer study, groups of rats and mice were given 2,4-TDA in diet for 103 weeks. Initial exposure levels for the rats were 125 and 250 ppm in feed, but because of low weight gain these amounts were reduced to 50 and 100 ppm after 40 weeks. Elevated mortality was seen in the high-dose group from 80 weeks onward. The histological findings included liver damage and kidney changes (32). Exposure levels for the mice were 100 and 200 ppm (≈15 and 30 mg/kg body weight/day), and liver hyperplasias and lower weight gain were observed in both exposure groups (32).

2,6-TDA

The lethal single dose with oral administration was 3000 mg/kg body weigbt for male rats and 1000 mg/kg b.w. for female rats (33). Rats exposed to 2,6-TDA in diet (> 1000 ppm) for 14 days lost weight. There was elevated mortality among mice given a diet containing 3000 ppm 2,6-TDA for 14 days. No effect was seen on body weights of either rats or mice after 14 days on a diet containing 300 ppm (33).

In a 13-week study, rats and mice were given 2,6-TDA in diet. Lower weight gain was noted in male rats at 100 ppm (the lowest dose) and in female rats at 1000 ppm. This effect was also noted in mice, at 300 ppm for males and at 1000 ppm for females: 100 ppm had no effect on weight gain in the mice (33). The rats developed thyroid hyperplasias at exposures of 3000 ppm or higher. Renal hyper-pigmentation and a papilloma in the forestomach were observed in the mice at

1000 ppm (33). In a cancer study, rats were given 250 or 500 ppm and mice 50 or 100 ppm 2,6-TDA in diet for 103 weeks. Lower weight gain was registered for the male rats in the high-dose group, the female rats in both dose groups, and the female mice (33).

Genotoxicity

2,4- and 2,6-TDA have been found to be equally mutagenic in several *in vitro* tests, including Ames' tests. For a more detailed review of *in vitro* tests, reference is made to the GDCH and IPCS documents (15, 23).

2,4-TDA

In vivo genotoxicity studies of 2,4-TDA have yielded positive results, especially with high doses. A dose-dependent increase of single-strand breaks was observed in the livers of rats after intraperitoneal administration of 2,4-TDA (37 – 500 mg/kg) (5, 22), although oral administration (50 or 150 mg/kg) had negative results (24). An increase of unscheduled DNA synthesis was noted in rat hepatocytes after oral administration of 2,4-TDA (150 mg/kg) (16, 30). Intraperitoneal administration of 2,4-TDA (9 or 18 mg/kg) increased sister chromatid exchange in the bone marrow of mice (35). There have been some studies on the ability of 2,4-TDA to produce chromosome damage (15). Elevated frequencies of micronuclei or chromosome aberrations have been seen in laboratory rodents only after high doses. 2,4-TDA induced mutations in Big Blue™ transgenic mice (19, 38).

2,4-TDA given to rats induces dose-dependent increases in DNA adducts, especially in liver and mammary glands. Three different types of DNA adducts were identified, and 60% of the adducts remained two weeks later (25). The lowest dose that induced DNA adducts in liver was 0.5 mg/kg (single dose, i.p.) (4, 25). In another study, it was shown that an oral dose of 50 mg/kg yielded greater numbers of adducts and more persistent adducts when administered over 10 days than when given all at once (27). The authors regard this as an indication that activation of 2,4-TDA was saturated. It has been demonstrated that the reactive metabolites are formed via P-450-dependent N-hydroxylation and sulfation (2, 12, 13, 20), and the mutagenic intermediate of 2,4-TDA is believed to be 4-acetoxyamino-2-aminotoluene (9). In a subsequent study with rats, adducts were identified 6 months after 6 weeks of exposure to 40 or 180 ppm 2,4-TDA in feed (11). In rats, 2,4-TDA has been shown to form covalent bonds to DNA, RNA and microsomal protein, and to the microsomal fraction of liver (12, 15).

2,6-TDA

For 2,6-TDA, most *in vivo* mutagenicity studies have been negative. Positive results are associated with extremely high doses (1).

2,6-TDA has been shown to give rise to hemoglobin adducts (34, 47, 49), although no DNA adducts could be detected in rat liver after intraperitoneal administration (150 mg/kg) (26). Both 2,4-TDA and 2,6-TDA have been shown to form hemoglobin adducts in rats (single doses of 0.5 to 250 mg/kg, i.p.), but

only 2,4-TDA induced DNA binding. When compared with 2,4-TDA, 2,6-TDA showed about one third as much binding to hemoglobin and plasma protein, and one third the tissue concentrations in liver and other organs (34).

Carcinogenicity

There are several older studies in which toluene diamines were tested for carcinogenicity. These results have been summarized by the IARC (22), and the IARC has classified 2,4-TDA as carcinogenic (Group 2B). In a later NTP cancer study (32), groups of 50 rats were given 125 or 250 ppm 2,4-TDA dihydrochloride in diet for 40 weeks. Levels were then reduced to 50 and 100 ppm due to toxicity, and animals in the low-dose group were exposed for a further 63 weeks. Due to the high mortality in the high-dose group, the animals were killed after 39 and 44 weeks. Dose-dependent increases were observed in the numbers of animals (males and females) with hepatocellular carcinomas. An increase in the number of animals with non-neoplastic changes was also noted. There were also elevated numbers of females with carcinomas or adenomas in the mammary glands and males with subcutaneous fibromas. In the same study, groups of mice were exposed to 100 or 200 ppm 2,4-TDA in diet for 103 weeks. A dose-dependent increase in hepatocellular carcinomas was seen in the females, and there was also an elevated frequency of lymphoma among females in the low-dose group. No significant increase of tumors was observed in the male mice (32).

2-6-TDA was tested in the same way (33). The dose levels tested were 200 and 500 ppm for rats and 50 and 100 ppm for mice. No significant increases in tumors were observed.

2,4-TDA (25 mg/kg/day, p.o., 30 days) increased the growth of pre-neoplastic foci in rat livers, whereas 2,6-TDA (50 mg/kg p.o., 30 days) was negative (39).

The difference in carcinogenic effect between 2,4-TDA and 2,6-TDA has been discussed in the literature. No differences in absorption or metabolism have been found that might explain it (8). The two substances have been found to be equally mutagenic in several different *in vitro* tests. Results of *in vivo* mutagenicity tests showed some differences, however, and 2,6-TDA was usually negative. Exposure to 2,4-TDA in doses about the same as those used in the NTP study has also been shown to increase cell proliferation in rat liver (10).

Teratogenicity

There have been several epidemiological studies addressing the question of whether mixed exposure to toluene diamine and dinitrotoluene has teratogenic effects. Teratogenic effects were found, but because of the mixed exposure these studies are of no value in assessing the teratogenicity of 2,4-TDA (15).

Effects on spermatogenesis have been documented in several animal studies with 2,4-TDA. They are ascribed to damage to Sertoli’s cells. In one study, rats were given 2,4-TDA (15 mg/kg/day) in diet for 10 weeks. The treatment induced degenerative changes in Sertoli’s cells and reduced spermatogenesis (48). In

another study with rats, the same exposure resulted in lowered testosterone levels and reduced spermatogenesis. The NOEL in this study was 5 mg/kg/day given in diet for 10 weeks (40). The NOEL was the same for the fertility index of male rats in another study (41). Mice were exposed to 2,4-TDA in a dominant-lethal test (40 mg/kg i.p. or p.o for 2 days), and no effect on fertility was observed (36). A dose of 150 mg/kg/day given to pregnant mice by gavage on days 7 to 14 of gestation had teratogenic effects, but the dose was too toxic to the mothers to allow the teratogenicity to be assessed (15). 2,6-TDA has not been tested for teratogenicity.

Dose-response / dose-effect relationships

There are no data on which to base a dose-response or dose-effect relationship for occupational exposure to 2,4-TDA or 2,6-TDA. The relationships between dose, response and effect observed in laboratory animals are summarized in Table 1 (2,4-TDA) and Table 2 (2,6-TDA).

Conclusions

There are no studies of the effects of TDA on human health. In animal experiments, the critical effect of 2,4-TDA is cancer. 2,4-TDA is a potent carcinogen for experimental animals, with the liver as the primary target organ. Dose-dependent increases of hepatocellular carcinomas have been observed in rats and female mice. 2-4-TDA also affects the reproductive systems of male rats. Both 2,4-TDA and 2,6-TDA are genotoxic.

There is no information that can be used to establish a critical effect for occupational exposure to 2,6-TDA. 2,6-TDA has shown no carcinogenic effect in animal experiments.

Table 1. Effects observed in experimental animals exposed to 2,4-TDA. (NOEL = No Observed Effect Level; c = control group; ld = low-dose group; hd = high-dose group).

Exposure	Species	Effect	Ref.
in diet, 7 weeks			
1000 ppm (75 mg/kg/day)	rat	Reduced body weight, increased hematopoiesis, changes in liver	32
250 ppm	rat	NOEL	
200 ppm	mouse	NOEL	
in diet, 103 weeks			
125 or 250 ppm. 40 weeks; doses then reduced to	rat	Reduced weight gain	32
50 or 100 ppm, (5.9 or 13 mg/kg/day) 63 weeks (ld) and 39 or 44 weeks (hd)		Reduced weight gain, elevated mortality (hd); hepatocellular carcinomas in females (0/20 c; 0/50 ld; 6/49 hd) and males (0/20 c; 5/49 ld; 10/50 hd) Mammary carcinomas or adenomas in females (1/20 c; 38/50 ld; 41/50 hd) Subcutaneous fibromas in males (0/20 c; 15/30 ld; 19/50 hd)	
100 or 200 ppm (15 or 30 mg/kg/day)	mouse	Lower weight gain, dose-related increase of hepatocellular carcinomas in females (0/19 c; 13/47 ld; 18/46 hd) Lymphomas in females in low-dose group (2/19 c; 29/47 ld; 11/46 hd)	32
p.o., single dose			
150 mg/kg	rat	Unscheduled DNA synthesis in hepatocytes	16, 30
p.o., 14 days			
100 mg/kg/day	mouse	Elevated liver weights	6, 46
25 mg/kg/day		Immunological effects, changes in hepatic enzymes	
i.p., single dose			
37 mg/kg	rat	Single-strand DNA breaks in liver	5, 22
p.o., 30 days			
25 mg/kg/day	rat	Increase of pre-neoplastic foci in liver	39
in diet, 10 weeks			
15 mg/kg/day	rat	Degenerative changes in Sertoli cells; reduced spermatogenesis; lower testosterone levels	40, 48
5 mg/kg/day	rat	NOEL	
i.p. single dose			
10 mg/kg	cat	Heinz inclusion bodies	37

Table 2. Effects observed in experimental animals exposed to 2,6-TDA (NOEL = No Observed Effect Level)

Exposure	Species	Effect/response	Ref.
in diet, 14 days			
>1000 ppm	rat	Weight loss	33
300 ppm		NOEL	
in diet, 13 weeks			
3000 ppm (225 mg/kg/day)	rat	Elevated mortality, thyroid hyperplasia	33
1000 ppm	rat	Reduced weight gain (females)	
1000 ppm	mouse	Renal hyperpigmentation, papilloma in forestomach. Reduced weight gain (females)	
in diet, 103 weeks			
500 ppm (24 mg/kg/day)	rat	Reduced weight gain (both sexes)	33
250 ppm (12 mg/kg/day)	rat	Reduced weight gain (females)	
in diet, 13 weeks			
100 ppm (7.5 mg/kg/day)	rat	Reduced weight gain (males)	33
100 ppm (15 mg/kg/day)	mouse	NOEL	
in diet, 103 weeks			
50 or 100 ppm (4.7 or 9.4 mg/kg/day)	mouse	Reduced weight gain (females)	33

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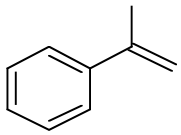
Consensus Report for α-Methylstyrene

November 1, 2000

Physical and chemical data. Uses

CAS No.:	98-83-9
Synonyms:	2-phenylpropene isopropyl benzene 1-methyl-1-phenylethylene 2-phenylpropylene α-methylstyrol 1-phenyl-1-methylethylene 1-methylethenylbenzene
Formula:	C_9H_{10}
Structure:	
Molecular weight:	118.19
Boiling point:	165 °C
Melting point:	- 23.2 °C
Vapor pressure:	0.307 kPa (20 °C)
Flash point:	54 °C
Density:	0.91 g/cm^3
Conversion factors:	1 ppm = 4.82 mg/m^3 1 mg/m^3 = 0.21 ppm

At room temperature α-methylstyrene is a clear liquid with a characteristic odor (odor threshold: 0.1 mg/m^3). It is soluble in benzene, diethylether, chloroform, acetone and carbon tetrachloride (in acetone and carbon tetrachloride in any proportions). It has an affinity for oils and fats (log $P_{o/w}$ = 3.34). Some t-butyl catechol may be added to α-methylstyrene to prevent peroxide formation during storage. Contact with heat or catalysts may cause α-methylstyrene to polymerize. A common use for α-methylstyrene is as a solvent and polymer in plastic production. It occurs in waxes, paints and other surface coatings.

Uptake, biotransformation, excretion

Both pure α-methylstyrene and α-methylstyrene emulsified in water can be absorbed through human skin. Undiluted α-methylstyrene (0.1 ml) spread on a measured glass surface was pressed against the skin of 8 volunteers for 8 to 10 minutes. Both the glass and the skin were then washed with alcohol, and the α-methylstyrene in the alcohol used for washing was determined with spectrophotometry (244 nm). The absorption rate for α-methylstyrene in water emulsion was determined by having 8 volunteers place their hands in 1-liter beakers of emulsion. After 1 hour of exposure the amount absorbed was determined by spectrophotometry. For undiluted α-methylstyrene, the absorption rate was 19.5 mg/cm^2/hour. For α-methylstyrene in water the absorption rate varied with concentration and temperature, and was 50% higher at 36 °C than at 24 °C (2).

Metabolism of α-methylstyrene is like that of styrene, its structural analogue. It is activated by P-450 monooxygenase to α-methylstyrene oxide (7-methylstyrene-7,8-oxide), and this metabolite is then transformed to α-methylstyrene glycol and various glutathione conjugates. Oxidation of α-methylstyrene glycol yields α-hydroxy-α-phenylpropionic acid (9), which has been identified in the urine of persons exposed to α-methylstyrene by inhalation and animals exposed by inhalation and intraperitoneal injection (1, 4, 5).

Toxic effects

Human data

Subjects (number not given) in an odor and irritation study were briefly exposed (duration of exposure not given) to α-methylstyrene vapors in a test chamber. At air concentrations of 2892 mg/m^3 (600 ppm) or higher they reported a very strong odor and experienced severe irritation of eyes and nose. A concentration of 482 mg/m^3 (100 ppm) had a strong odor but did not cause excessive discomfort. At an air concentration of 241 mg/m^3 (50 ppm) the subjects reported a pronounced odor but no irritation (15).

A change in the light sensitivity of the eye was observed in three odor-sensitive persons after 15 to 20 minutes of exposure to a concentration of 0.1 mg/m^3 (0.02 ppm) α-methylstyrene (odor threshold 0.1 mg/m^3). These exposure conditions also produced changes in the α-rhythm on their EEGs. Neither effect was observed at 0.08 mg/m^3 or 0.04 mg/m^3 (10). These effects are not considered to be toxicologically relevant.

There is a study describing a correlation between clinical symptoms of poisoning and deterioration of renal circulation in 69 occupationally exposed subjects employed in the production of divinyl-α-methylstyrene rubber. The symptoms appeared after seven to eight years of employment. No further details of this study were available (8). A case report describes workers handling divinyl-α-methylstyrene rubber who developed ‘neurocirculatory dystonia’ as a result of occupational exposure to 1,3-butadiene and α-methylstyrene. They also had some

deterioration in liver function, but the cause of this is unclear. Further information was not available (3).

Animal data

Effects of α-methylstyrene on skin were examined in a study with rabbits: undiluted α-methylstyrene was painted on their ears and shaved rumps (10-20 strokes spaced over 2 to 4 weeks). The treatment resulted in moderate to marked erythema as well as some necrotic effect in the form of exfoliation. In another study, undiluted α-methylstyrene (two drops) was applied to the eyes of rabbits and the eyes were examined after 3 minutes, 1 hour, 24 hours, 2 days and 7 days. The substance caused slight inflammation of the conjunctiva, but no visible damage to the cornea (15).

The LD_{50} for rats given α-methylstyrene per os was 4.9 g/kg. Liver damage was found when the animals were dissected (15).

Mice were exposed to α-methylstyrene in air (2900, 3860 or 4800 mg/m^3) 6 hours/day for 12 days. Mortality for the females was 6% (1/18) at 2900 mg/m^3, 56% (10/18) at 3860 mg/m^3, and 21% (5/24) at 4800 mg/m^3. The cause of death could not be determined. No male mice died during the exposures. During the first hour of exposure the mice in all exposed groups were hyperactive and did not react to noise. By the end of the 6-hour exposure the animals had calmed down and appeared drowsy. The mice adapted to this 'tranquilizing' effect of α-methylstyrene after a week of exposure (5 days/week). Higher liver weights and lower spleen weights were found in both males and females in all exposed groups. Liver weights were significantly higher in the males after a single 6-hour exposure to 3860 mg/m^3, and in the females after 5 days of exposure to 2900 mg/m^3. After 5 days at 3860 mg/m^3 reduced spleen weights were seen in both sexes. Only the male mice had significantly lower body weights after 5 days of exposure. Histopathological examinations revealed no exposure-related tissue changes. After 1 and 5 days of exposure there was a reduction of glutathione in the liver in all three exposed groups (2900, 3860 and 4800 mg/m^3) (9). In a short-term test, male mice that inhaled α-methylstyrene had a higher threshold for seizures induced by pentetrazole. A concentration of 3581 mg/m^3 (743 ppm) α-methylstyrene caused a 50% seizure threshold increase (STI_{50}) for pentetrazole (6).

In an inhalation study, exposure to 2900 or 4800 mg/m^3 α-methylstyrene 6 hours/day for 12 days resulted in higher liver weights in F-344 rats. Hyalin drop formation could be observed in the kidneys of F-344 males after nine days of exposure to 1205 mg/m^3 but could not be observed in F-344 females or male NBR rats (which lack α2u-globulin production) under the same exposure conditions. The effect is considered to be related to α2u-globulin, a protein that occurs in much greater concentrations in male rats than in female rats. There was no hyalin drop formation in the F-344 males exposed to 603 mg/m^3 (9).

Histological examination of male white rats exposed to 5 mg/m^3 α-methylstyrene 24 hours/day for 3 months revealed interstitial pneumonia, bronchial inflammation (desquamative bronchitis), abnormal growth (hyperplasia) of

bronchial lymph nodes, tissue changes in the walls of blood vessels, degenerative changes in kidneys and heart muscles, disturbances of glycogen-related functions in the liver (not explained) and neural cell dystrophy in the cortex, cerebellum and spinal cord. After 2 weeks of exposure to 5 mg/m^3 the rats had lower coproporphyrin levels in urine (not further elucidated) and a higher number of leukocytes that showed abnormal coloration (green to yellow/yellow-orange) when dyed with acridine orange (not explained). Only minor changes were observed in lungs, liver and acridine-orange dyed leukocytes of rats exposed to 0.5 mg/m^3 for 3 months, and no effects were observed in rats exposed to 0.05 mg/m^3 for 3 months (10). Data in this study are inadequately presented and 'end points' are unclear.

In an inhalation study (15), Wistar rats (10 to 25 per group, both sexes) were exposed to α-methylstyrene concentrations of 970 mg/m^3 (for 139 7-hour sessions), 2900 mg/m^3 (for 149 x 7 hours), 3860 mg/m^3 (28 x 7 hours) or 14,490 mg/m^3 (for 3 or 4 x 7 hours) (= 200, 600, 800 or 3000 ppm). The exposure to 14,490 mg/m^3 resulted in high mortality (not quantified). The group exposed to 3860 mg/m^3 lost weight, and kidney and liver weights were affected (no quantitative data given). Effects on kidney and liver weights were also observed at 2900 mg/m^3. No effects were observed at 970 mg/m^3.

Guinea pigs were exposed according to the same protocol. Those exposed to 3860 mg/m^3 lost weight, and liver and kidney weights were affected. Liver weight was affected at 2900 mg/m^3. No effects were seen at 970 mg/m^3. Rabbits were exposed to 970 mg/m^3 (139 x 7 hours) or 2900 mg/m^3 (149 x 7 hours). The lower dose group showed no effect. In the higher dose group the exposure resulted in higher mortality and weight loss. Rhesus monkeys (1 or 2) of both sexes were exposed along with the rabbits, and neither exposure level resulted in observable histopathological changes or effects on organ weight (15).

Mutagenicity, carcinogenicity, teratogenicity

Ames' tests with α-methylstyrene were negative, both with and without metabolic activation (16), although the intermediate metabolite α-methylstyrene oxide has yielded positive results (14). Sister-chromatid analysis of human lymphocytes *in vitro* revealed a significant increase in the frequency of sister chromatid exchanges (11). Unlike styrene, which is activated by hemoglobin, α-methylstyrene's ability to induce sister chromatid exchanges is not dependent on erythrocytes (11, 12, 13).

Methylstyrene (unspecified isomer) was tested for teratogenicity in a study with rats: 250 mg/kg body weight (in corn oil) was injected intraperitoneally. The treatment had no observed effect on the mothers, but there were changes in the sex ratio of the embryos (fewer females) and a significantly higher frequency of resorptions (7).

Table 1. Some effects observed in laboratory animals exposed by inhalation to α-methylstyrene.

Exposure level (mg/m^3)	Duration	Species	Effect	Ref.
970	139 x 7 hours	rat (Wistar)	NOEL	15
2900	149 x 7 hours	rat (Wistar)	Changes in kidney and liver weight LOEL	15
2900	6 hours	mouse ($B_6C_3F_1$)	Glutathione reduction in liver LOEL	9
2900	12 x 6 hours	rat (F344)	Elevated liver weight	9

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

Dose-response / dose-effect relationships

Brief exposure to 482 mg/m^3 (100 ppm) α-methylstyrene in air produced symptoms of irritation in eyes and respiratory passages of subjects in an exposure chamber (LOEL). At 241 mg/m^3 (50 ppm) the odor was apparent but no symptoms of irritation were noted (NOEL). There are no other data on human exposures that can be used as a basis for describing a dose-effect or dose-response relationship. Dose-response and dose-effect relationships observed in animal experiments are given in Table 1. Preliminary data indicate that α-methylstyrene may be fetotoxic.

Conclusion

The critical effect of exposure to α-methylstyrene is irritation of eyes and mucous membranes These effects were observed in subjects briefly exposed to 482 mg/m^3 (100 ppm) in a test chamber, but this study provides no information on which to base a NOEL for occupational exposure to α-methylstyrene.

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Consensus Report for Hydrogen Cyanide, Sodium Cyanide and Potassium Cyanide

February 7, 2001

Chemical and physical data. Uses

hydrogen cyanide

CAS No.:	74-90-8
Synonyms:	hydrocyanic acid, prussic acid, formonitrile
Formula:	HCN
Molecular weight:	27.04
Boiling point:	25.70°C
Melting point:	-13.24°C
Vapor pressure:	83.8 kPa (20°C)
Saturation concentration:	827,246 ppm
Conversion factors:	1 mg/m^3 = 0.89 ppm (20°C) 1 ppm = 1.12 mg/m^3 (20°C)

sodium cyanide

CAS No.:	143-33-9
Synonyms:	cyanide of sodium
Formula:	NaCN
Molecular weight:	49.01
Boiling point:	1496°C
Melting point:	563.7°C
Vapor pressure:	-

potassium cyanide

CAS No.:	151-50-8
Synonym:	cyanide of potassium, cyanide of potash
Formula:	KCN
Molecular weight:	65.12
Boiling point:	-
Melting point:	634.5°C
Vapor pressure:	-

HCN occurs as a colorless gas or as a clear, extremely volatile and flammable liquid. It has an odor of bitter almonds. It is soluble in ethanol and similar solvents and mixes with water, forming a weak acid (4, 44). One source reports an odor threshold of 0.2-5 ppm for HCN (21), but there are many people who can not perceive the odor at all (39, 40). NaCN and KCN occur as clear or whitish solids (4). They are readily soluble in water (about 50 g/100 ml cold water), producing strongly alkaline solutions (59, 68).

Occupational exposure to cyanide can occur during industrial production and use of cyanide compounds, and may also result from exposure to industrial chemicals that are metabolized to cyanide, such as simple aliphatic nitriles (4). Cyanides also occur naturally in many foods (e.g. cassava/manioc, passionfruit, bamboo shoots, bean sprouts and almonds, and the pits of apricots, peaches, cherries and plums) in the form of cyanogenic glycosides, which can be broken down to HCN in the intestine (4, 34, 46). Cyanide may occur in foods as pesticide residue (4, 34). Cyanide exposure may also have several other sources, including tobacco smoke, smoke from fires, vehicle exhausts and some medicines (4, 34).

Cyanides are used primarily in the steel, mining, and chemical industries and in electroplating (4). NaCN is used, for example, to extract gold and silver from ore and in the production of adiponitrile (for nylon), pigments, chelating agents and pesticides (34, 40). NaCN and KCN are used together to treat steel. Large amounts of cyanide salts such as NaCN and KCN are used in electroplating – KCN particularly with silver plating (4, 34). Many metal polishes contain NaCN or KCN. HCN is used in the production of adiponitrile (for nylon), methylmeth-acrylate, NaCN, chelating agents, and pharmaceuticals. HCN may also be used to fumigate grain storage facilities to kill rodents and insects (4).

Uptake, biotransformation, excretion

HCN, NaCN and KCN are rapidly absorbed in the intestine (4, 6, 73). HCN is also taken up very rapidly by the lungs (4, 6). Monkeys were exposed by inhalation to 100-150 ppm HCN, and a constant blood level was reached within about 10 minutes (67). In an older study, volunteers were exposed to 0.5-18 ppm (0.5-20 mg/m^3) HCN by breathing through the mouth. Samples were taken from the start of exposure for up to about 3 minutes, and lung retention was calculated to be about 40-80% (about 60% with ‘normal’ breathing). The results were not concentration-dependent (52). When volunteers were exposed to 1-10 ppm (1.1-11 mg/m^3) HCN by breathing through the nose, retention was reported to be 13-23% regardless of concentration (52). Uptake of NaCN and KCN via the lungs is probably also quite high, but no studies or estimates were found.

Skin uptake varies. In a study with rabbits, application of NaCN dust to dry, intact skin was reported to result in absorption of amounts too small to produce indications of systemic toxicity. Damp NaCN (paste), however, and HCN, NaCN or KCN in solution, were rapidly absorbed through intact skin in amounts sufficient to cause deaths (6). *In vitro* studies (20) have revealed that the

absorption rate of NaCN in aqueous solution is strongly dependent on pH in the interval 9-12. The steady-state absorption rates of CN (pH 11.2-11.4) with application of a 1%, 10%, or 40% solution of NaCN were reported to be 2.3, 58 and 62 µg/cm^2/hour, respectively. These studies also showed that HCN is rapidly absorbed through the skin. The reported permeability constant for the cyanide ion was 3.5 x 10^4 cm/hour, and that for HCN 100 x 10^4 cm/hour (in aqueous solution). This means that, at steady-state, HCN is absorbed 30 times faster than CN (20).

After uptake, cyanide is reversibly bound to the methemoglobin in red blood cells and efficiently distributed by the blood throughout the body (4, 14, 73). The total binding capacity of methemoglobin at normal physiological levels has been reported to be about 8 mg HCN (73). The cyanide that is not bound to methemoglobin is metabolized in various tissues, notably the liver, kidneys and nasal epithelium (6, 12, 14, 31, 55). Several biotransformation pathways have been identified (Figure 1). The main one involves enzymatic transsulfurization to thiocyanate (6, 46, 88). The detoxification rate in humans after intravenous administration of HCN is reported in one work to be 0.017 mg CN/kg body weight/minute (McNamara 1976, cited in 6; 24, 53). In a pharmacological paper, however, it is reported that the maximum detoxification rate for cyanide in humans is only 0.6 to 0.9 µg/kg body weight/ minute, and that the earlier assumption that the human body can transform 0.017 mg CN/kg body weight/minute is erroneous (74). This author also reports that the detoxification rate is considerably lower in humans than in laboratory rodents or dogs (73). Access to a sulfur substrate (especially thiosulfate) usually determines the speed and efficiency of the transformation of cyanide to thiocyanate (6, 12, 31, 46, 74). It has been reported that metabolism of cyanide is affected by nutrient imbalances, especially protein deficiency (13, 74, 82).

Most absorbed cyanide is excreted in urine, primarily as thiocyanate, but small amounts are also eliminated in urine and exhaled air as HCN, carbon dioxide and other biotransformation products (6, 56, 60, 90). The average half time for excretion of thiocyanate has been reported to be 2.7 days in healthy subjects and 9 days in subjects with renal insufficiency (73). The portion excreted in human urine as thiocyanate has been found to be many times larger than the portion detected in urine as cyanide, with both ‘normal’ exposure (via food, tobacco smoke etc.) and occupational exposure (15, 57). Smokers excrete much more thiocyanate in urine than non-smokers do (15).

A correlation between air concentration of cyanide and excretion of thiocyanate in urine was determined in a study: the amount of thiocyanate in urine (24-hour samples) = 0.65 x air concentration of cyanide (ppm) (22). “Air concentration” refers to the average concentration of cyanide in the air for the last three days of the work week, and the correlation applies to the average concentration of thiocyanate in urine during the same period.

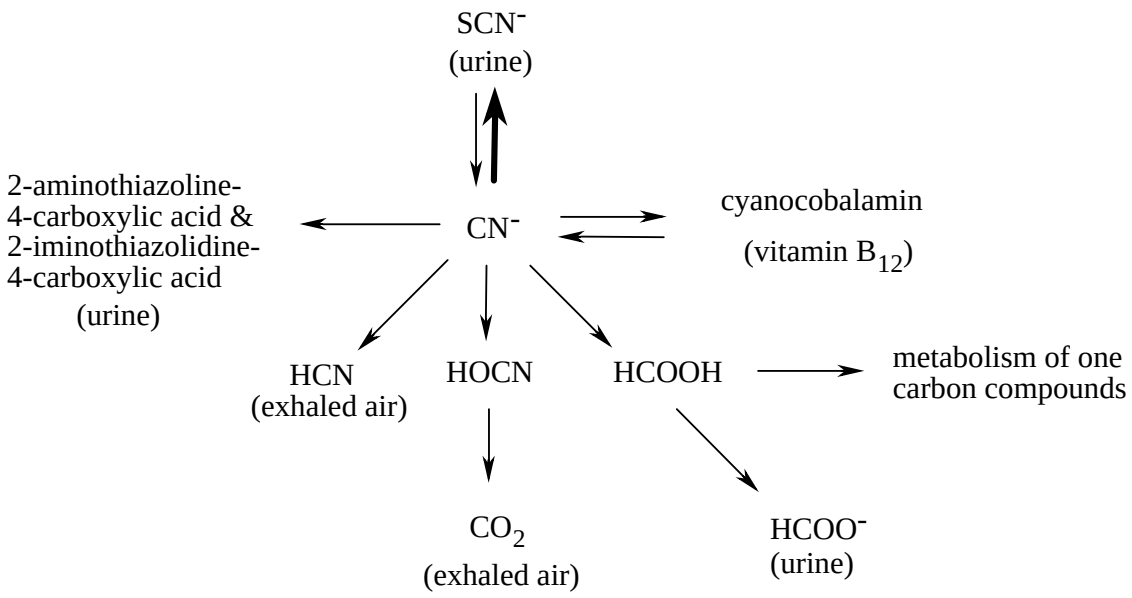

Figure 1. The primary metabolic pathways for cyanide (adapted from Reference 3).

Toxic effects

Human data

In cases of acute cyanide poisoning the primary target organs are the central nervous system and the heart (4). The toxic effects of cyanides are attributed primarily to the (reversible) formation of complexes with the Fe^3 ion in the enzyme cytochrome c oxidase, which compromises the cells' ability to exploit oxygen (4, 6, 8, 30, 34, 39). Cyanides also inhibit many other enzyme systems, however, and these reactions contribute to the classic symptoms of acute cyanide poisoning (4, 6). The indications of acute cyanide poisoning include rapid breathing, dizziness, confusion, headache, nausea, vomiting, atony, ataxia, dyspnea, cardiac arrhythmia, convulsions, coma and death (32, 34, 64, 89). With lower exposures, symptoms of acute cyanide poisoning may appear after accumulation of cyanide (6).

There are numerous reported cases of acute and sometimes fatal poisoning due to oral intake of cyanides in various contexts. The lowest fatal dose (absorbed dose) for oral intake of single doses by humans has been calculated to be about 0.5 mg HCN/kg body weight (29) . Acute poisoning has also been observed after skin exposure to very high air concentrations of HCN or to HCN in liquid form (20, 65, 75). In one case, dizziness, breathing difficulty and loss of consciousness were reported within three to five minutes after HCN had been spilled on the patient's hand (65). Deaths have been reported following acute inhalation exposure to relatively high air concentrations of HCN (see Table 1).

Systemic effects, notably on the nervous system, digestive tract and thyroid, have been reported after repeated occupational exposure to cyanides (7, 11, 22, 32, 72). In some cases the symptoms involving the nervous system and digestive

tract took some time to appear, and it is not clear whether this represents a chronic form of cyanide poisoning or acute effects. Thyroid hypertrophy has been associated with chronic exposure to cyanides and seems to be related to the thiocyanate ion (4, 33). Cyanides are also suspected of contributing to the deterioration of vision observed in some smokers (tobacco amblyopia), especially those with high alcohol consumption and nutritional deficiencies (26, 27, 70, 78).

Neurological effects and effects on the thyroid have been documented after prolonged consumption of foods containing cyanogenic glycosides. Outbreaks of konzo, a distinct form of tropical myeloneuropathy characterized by abrupt onset of incapacitating spasms, have been reported in populations in Africa after periods when the diet consisted mostly of cassava. Another cassava-related neural disease is tropical atactic neuropathy. It usually develops gradually over the course of several years (41, 62, 79, 82, 83). Another disease that has been associated with high and prolonged cassava intake is goiter, which appears primarily in conjunction with low iodine intake (1, 2, 16).

In a retrospective study made in the United States, symptoms of cyanide poisoning were seen in factory workers (n = 36) who had been exposed to cyanide (NaCN, HCN) during a silver reclaiming process (11). The workers had been exposed to cyanides via inhalation, skin contact and possibly also oral intake. The average air content of HCN one day after the factory had been closed (24-hour measurement) was 15 ppm. More than two thirds of the workers reported on a questionnaire that they had experienced headaches, dizziness, nausea/vomiting, and/or a bitter taste in the mouth on more than 10 occasions per month. Other reported symptoms included eye irritation, loss of appetite, weight loss, nose-bleeds, fatigue, skin rashes, heavy perspiration, shortness of breath, cough, sore throat, chest pains, changes in the sense of smell, coughing up blood, heart palpitations and fainting. There was a correlation (significant positive trend) between exposure level and the subjects' assessments of the severity of the poisoning, but the exposure estimate was not connected to the air measurements. Some symptoms were reported to last for seven or more months after exposure had been terminated (lower prevalence). Biochemical analyses made more than half a year after exposure had stopped revealed somewhat higher average values of thyroid-stimulating hormone (TSH) in serum (high-exposure group 2.4 µU/ml, laboratory control average 1.7 µU/ml) and somewhat fewer free bonding sites on thyroxine-binding globulin (TBG) in serum (T3U test: high-exposure group 32.4%, laboratory control average 30.0%). On the other hand, values for the thyroid hormone T_4 (thyroxine) were normal and there were no palpable changes in thyroids. The exposed workers (particularly those with high exposure) also had lower average levels of vitamin B_{12} and folic acid in serum. The results are interpreted by the authors as possible indications of effects of long-term cyanide exposure (11).

In another epidemiological study, 36 Egyptian workers (non-smokers) who had been exposed to cyanide for 5 to 15 years of working with a plating bath containing 3% NaCN and 3% copper cyanide were compared with 20 controls (22). Head-

aches, weakness, changes in the senses of taste and smell, dizziness, throat irritation, vomiting, shortness of breath with physical exertion, watery eyes, chest pains and several other symptoms were reported more frequently by the exposed workers than by controls. Two of the nine workers from the factory with the highest exposure levels (8.2-12.4 ppm) were reported to have suffered psychotic episodes. Slight to moderate thyroid enlargement was seen in 20 of the exposed workers, but none of them had clinical indications of hypo- or hyperthyroidism. Changes in blood profile, including significantly higher hemoglobin levels and lymphocyte counts, were also noted in the exposed workers. The workers were given radioactively labeled iodine after two days away from work, and much higher iodine concentrations ($p < 0.001$) were found in the thyroids of the cyanide-exposed workers than in controls [average values for exposed workers: 38.7% (4 h), 49.3% (24 h); averages for controls, 22.4% (4 h), 40% (24 h)]. There was no significant difference for iodine bound to protein in blood (^{131}PBI) after 72 hours. Air concentrations of cyanide in the breathing zone of exposed workers (15-minute samples) ranged from 4.2 to 12.4 ppm (average concentrations 6.4-10.4 ppm). The concentration of thiocyanate in urine increased gradually, and during the last three days of the work week it was virtually constant and strongly correlated to the average air concentration of cyanide (22).

In an Indian study (7), serum from 35 workers who handled cyanide salts in a cable industry (electroplating) and 35 matched controls was analyzed for evidence of effects on thyroid function. All subjects were non-smokers. No information on air concentrations is given. The content of thiocyanate in serum was higher in the exposed group than in the controls. The exposed workers had significantly lower serum levels ($p < 0.05$) of thyroid hormones T_4 and T_3, and significantly higher levels ($p < 0.05$) of TSH, than the controls. In the exposed group there was also a significant positive correlation between serum levels of TSH and thiocyanate ($p < 0.05$) and a significant negative correlation between T_4 and thiocyanate ($p < 0.05$). According to the authors, these results indicate that occupational exposure to cyanide may inhibit thyroid function (7).

Another Indian study (15) reports that symptoms characteristic of cyanide poisoning were observed in 23 workers exposed to HCN gas and cyanide aerosol during case-hardening and electroplating operations. The workers had elevated levels of cyanide and thiocyanate in blood and urine, but no details on their other symptoms are given. The measured cyanide levels in the breathing zone were in the range 0.1-0.2 mg/m^3 (0.09-0.19 ppm), and measurements made on the shop floor yielded values in the range 0.2-0.8 mg/m^3 (0.19-0.74 ppm). The limited information in this report provides insufficient basis for any conclusions regarding a relation between effects on health and air concentrations of cyanide.

Significantly elevated serum values for zinc, calcium and iron were found in a study of women who reportedly were exposed to HCN and cyanides 8 hours/day at work (37). The same authors report in another study that significantly elevated serum values for L-aspartate-2-oxyglutarate-aminotransferase (SGOT) and lactate dehydrogenase (LDH) were found in women (probably at the same workplace)

who reportedly were exposed to vapors of HCN and cyanides 8 hours/day (38). In both studies, the authors interpret the findings as effects of cyanide exposure. It is also reported that on a few occasions the threshold limit of 5 mg HCN/m^3 was exceeded, air concentrations (calculated as HCN) then reaching a maximum of 7.5 mg/m^3. No details of the measurements are given, however. There is little information on other occupational exposures or any differences in food intake between the exposed group and controls, and the interpretation of the results must therefore be regarded as uncertain.

It is reported in a reference work (23) that a mist of an alkaline solution consisting mostly of NaCN caused severe nasal irritation and in some cases erosion of the nasal septum in occupationally exposed persons, and that the air concentrations of cyanide (expressed as HCN) had not been much above 5 ppm. It is suggested, however, that these persons may have been exposed to other alkaline substances that may have contributed considerably to the observed irritation.

Skin contact with NaCN or KCN can cause severe skin irritation as well as systemic toxicity (6, 40). The skin irritation is probably due to the alkalinity of the substances.

Animal data

HCN, NaCN and KCN are extremely toxic. With oral intake, the reported LD_{50} for all three substances is between 2.5 and 16 mg/kg body weight (rabbit, rat, mouse). For skin application of solutions (intact skin of rabbit), the reported LD_{50} is 6.9 mg/kg b.w. for HCN, 14.6 mg/kg b.w. for NaCN, and 22.3 mg/kg b.w. for KCN (6, 24). With inhalation exposure, the LC_{50} for HCN varies with species and exposure time, but in some studies (rabbit, rat, mouse) has been reported to be about 110-170 ppm for 30 minutes of exposure, about 320-500 ppm for 5 minutes of exposure, and about 1000-3400 ppm for exposures of 1 minute or less (6, 54, 58, 86).

Inhalation exposure to HCN affects most prominently the central nervous system (Table 2). Hyperventilation, grogginess and slow, irregular heartbeat, and in many cases cramps or apnea were observed in a study in which monkeys were exposed to 100-156 ppm HCN for up to 30 minutes. At an air concentration of 100 ppm it took 19 minutes for the animals to become so affected that they were unable to move (67). A slight dampening effect on the CNS was observed at an exposure to 60 ppm HCN (monkeys, 30 minutes). EEG changes (including increased delta and theta activity) and reduced amplitude in measurement of auditory cortical evoked potential could be observed toward the end of the exposure period. Some increase in respiratory minute volume was also noted. There were no significant effects on heart/vascular parameters, peripheral neuromuscular impulse transmission, or neural transmission speed (66). Reduced respiratory minute volume, respiratory rate and pulmonary compliance, and lower phospholipid levels in the lungs (surfactant), were reported in another study in which rats were exposed to 55 ppm HCN for 30 minutes. Increased transthoracic pressure, air flow and tidal volume were also observed (9). In a study with mice,

the DC_{50} (50% reduction of respiratory rate due to effects on the respiratory center) for 30 minutes of exposure to HCN was calculated to be 63 ppm. Intermittent sensory irritation was also observed (58).

A French study (85, cited in 4) reports observations of dyspnea, vomiting, diarrhea, tremor, spasms, ataxia and coma in dogs that had been exposed to 45 ppm HCN 30 minutes/day for 28 to 96 days. Histological changes in the brain were also observed. In an older study with several species, at exposure to 45 ppm HCN the animals fell and remained lying on their sides within 15 to 30 minutes (25). In a study with rabbits in which heart, lungs and associated arteries were studied, no treatment-related effects were reported after constant exposure to about 0.5 ppm HCN for up to 4 weeks (42, 43).

Effects on the central nervous system/behavior, thyroid, kidneys and reproductive organs have been reported after repeated exposure by means other than inhalation (Table 3). One study describes degenerative changes in the central nervous system and inflammatory changes in the digestive tract in two dogs given 0.5 or 2 mg NaCN (= 0.27 or 1.1 mg CN) /kg body weight/day in gelatin capsules for 13.5 or 14.5 months. Symptoms of acute toxicity were sometimes noted in conjunction with administration (36). In another study, pigs were given an aqueous solution of KCN by gavage for 24 weeks. Doses were equivalent to 0.4, 0.7 or 1.2 mg CN/kg body weight/day. There was a gradual reduction in blood levels of T_3 and to some extent also T_4 in all dose groups (especially the high-dose group), and progressive, dose-dependent increases of blood glucose (fasting values) and effects on behavior were also noted (45). Kidney damage (nephrosis), indications of hyperplasia/hypertrophy of adrenals (zona glomerulosa) and effects on testes (see Effects on reproduction) were observed in histopathological examination of dogs after 14 weeks on a diet containing NaCN in an amount equivalent to 1.04 mg CN/kg body weight/day (47). In earlier studies by the same author, dogs fed on this diet for 14 weeks developed hypothyroidism and goiter, and there were also effects on the pancreas (necrosis, fibrosis, atrophy) (48, 49).

In an NTP study (34), NaCN mixed in the drinking water of rats and mice in amounts ranging from 3 to 300 mg/l (rats: about 0.16-13 mg CN/kg b.w./day; mice: about 0.3-27 mg CN/kg b.w./day) for 13 weeks caused changes in various reproduction parameters (see Effects on reproduction). Other observations included lower water consumption, especially in the higher dose groups, but there were no clinically significant changes in body weight, organ weight, or hematological or clinical-chemical parameters, and no histopathological changes in examined organs (including thyroid, brain, adrenals, kidneys, liver, heart and lungs).

Cyanides can irritate the eyes. Local effects observed in eyes of rabbits after instillations of HCN, NaCN and KCN were inflammation, gradual swelling of the conjunctiva, and cloudiness of the cornea (5, 6). Severe symptoms of poisoning were also noted after concentrated solutions had been applied to the eyes (5).

Mutagenicity, carcinogenicity

HCN was mutagenic to the *Salmonella typhimurium* strain TA100, but not TA98. The mutagenic activity was reduced by metabolic activation (51). NaCN and KCN were reported to be non-mutagenic to strains TA97, TA98, TA100, TA1530, TA1535, TA1537, TA1538 and/or TA1950 both with and without metabolic activation (17, 18, 34, 50, 69). KCN, however, was positive in a DNA repair test with *E coli* (18). KCN induced chromosomal aberrations when tested on mammalian cells *in vitro*, and higher doses induced DNA fragmentation (10, 84). *In vitro* studies with human cells indicate that KCN is cytotoxic but not genotoxic (35, 87). Inhibited DNA synthesis, but no DNA damage, was reported in an *in vitro* DNA-synthesis inhibition test (KCN) using HeLa cells (63). However, inhibition of DNA synthesis (testes) was not observed *in vivo* after oral administration of 2.5 mg KCN/kg body weight to mice (28).

No studies specifically of carcinogenicity were found in the literature. In one study in which rats were given extremely high amounts of KCN in diet – 50 or 100 g/kg feed (sic!) – for 14 weeks (61), benign tumors were found in cecum and large intestine (7/10 animals in the high-dose group; 5/10 animals in the low-dose group; results in controls not reported). The tumors consisted of many hypertrophic muscle fibers with no indication of malignancy. Other observations included reduced body weights, lower relative spleen weights, higher relative thyroid weights, hepatic cell necroses and hematological /biochemical changes.

Effects on reproduction

Hamsters were given slow infusions of NaCN from subcutaneous implants liberating 6.18 to 6.35 mg/kg b.w./hour, equivalent to about 148-152 mg/kg b.w./day (total doses more than 30-40 times the LD_{50}), on days 6 to 9 of gestation. There were signs of toxicity in the mothers, especially at the higher dose, and high incidences of severe malformations and resorptions at all dose levels (19). An abstract (76) reports inhibited growth, meningoceles/myeloceles and some deaths in embryos of rats given daily intraperitoneal injections of KCN (aqueous solution) during the first 15 days of gestation (3 mg/kg b.w. per injection).

In an experiment in which rats were given NaCN by gavage (6 mg/kg b.w. in water) on day 10 of gestation, morphological anomalies were noted in some fetuses 48 hours after the administration. Clinical indications of toxicity (including deaths) were observed in the mothers (71). In studies in which pregnant pigs and rats (80, 81) were fed on cassava containing up to 1250 mg KCN/kg, the only observed toxic effects on reproduction were minor metabolic differences and lower relative organ weights in fetuses at doses affecting the mothers.

In an NTP study, mice and rats were given drinking water containing 30, 100 or 300 mg NaCN/liter (rats: about 1.6-13 mg CN/kg b.w./day; mice: about 3-27 mg CN/kg b.w./day) for 13 weeks, and examined for effects on various reproduction parameters (reproductive toxicity at lower doses was not studied). Effects were

particularly evident in the rats, but were judged by the authors to be "probably insufficient to decrease fertility in rats." Significant, dose-dependent reductions of cauda epididymal weight (all dose groups) and dose-dependent reductions (significant in the high-dose group) of epididymis and testis weights were observed in rats. Cauda epididymis and epididymis weights were significantly lower in the high-dose group of mice. The number of sperm per testis was significantly lower in the high-dose group of rats. Sperm motility was also lower in the rats (all dose groups), but this was judged to have no biological significance. Altered estrus cycles were observed in female rats in the two higher dose groups, but no effects on estrus cycle were seen in the female mice (34). Effects on spermatogenesis (significantly lower relative frequency of stage 8 tubuli and degenerative changes with elevated occurrence of abnormal gametes) were observed in testes of dogs given food containing NaCN (dose equivalent to 1.04 mg CN/kg b.w./day) for 14 weeks (47).

Dose-effect/dose-response relationships

There are few reliable measurements of air concentrations of HCN, NaCN or KCN in an occupational exposure context. In most of the studies that report air concentrations, exposure to cyanide by other means (such as skin uptake) can be suspected, and it is therefore difficult to determine dose-response relationships with certainty. There is the additional problem of mixed exposures. Irritation of respiratory passages and toxic symptoms indicating effects on the central nervous system have been reported in several studies, however. Effects on the thyroid have also been noted after exposure to HCN and cyanide salts, and have been presented as possible long-term effect of exposure. One work (22) reports that effects on the central nervous system (including two psychoses, headache, vomiting, dizziness, weakness, changes in the senses of taste and smell) were more common among exposed persons than among controls, and that the measured air concentrations of cyanide in the breathing zones of the exposed workers (15-minute samples) ranged from 4.2 to 12.4 ppm (average concentrations 6.4 to 10.4 ppm). Effects on the thyroid were also noted at these air concentrations (Table 1). There are no data indicating skin uptake of cyanide in this study.

The maximum detoxification rate for cyanide in humans, as calculated from information on infusions of medicine given to acutely ill patients, is about 60 µg CN/minute (74). Assuming that inhaled air amounts to 20 l/minute and uptake is 100%, this detoxification rate corresponds to uptake from inhalation exposure to about 3 mg/m^3. This calculation indicates that at higher exposure levels there may be an accumulation of cyanide in the body, and it can therefore be assumed that about 6 hours of constant exposure to 4 mg/m^3 might give rise to cyanide levels in the blood that can yield biochemically detectable metabolic disturbances.

Dose-effect relationships observed in animals experimentally exposed to HCN, NaCN and KCN are summarized in Tables 2 and 3. Effects on the brain have been

observed at daily oral doses of 0.5 mg NaCN/kg b.w. (equivalent to 0.27 mg CN/kg b.w.), and effects on behavior and/or thyroid hormones have been noted with daily oral doses of 1 mg KCN/kg b.w. (equivalent to 0.4 mg CN/kg b.w.) (36, 45). Lower doses were not tested. Effects on adrenals, kidneys, thyroid and spleen, as well as effects on spermatogenesis, have been reported with administration of somewhat higher dose levels of NaCN in food.

Conclusions

The critical effect of occupational exposure to cyanide is its effect on the central nervous system. Severe CNS effects have been reported after occupational exposure to air concentrations of 4 to 12 ppm cyanide (breathing zone). Some effects on thyroid have also been documented at these air concentrations. Similar toxic effects on the CNS and thyroid have also been reported in laboratory animals exposed to HCN, NaCN or KCN. Pharmacological studies indicate that at exposures to air concentrations around 3 ppm cyanide can accumulate in the body. Cyanide is rapidly absorbed through the skin and can cause acute and sometimes lethal poisoning.

Table 1. Dose-effect relationships reported in humans exposed to cyanide by inhalation.

Exposure	Effect	Ref.
270 ppm	Lethal (death within 6 to 8 minutes)	25
181 ppm	Lethal (death after 10 minutes)	30
135 ppm	Lethal (death after 30 minutes)	30
110-135 ppm	Highly dangerous/lethal (death within 30 to 60 minutes)	25
4.2-12.4 ppm* (average 6.4-10.4 ppm)	Headache, weakness, changes in sense of taste and smell, dizziness, throat irritation, vomiting, shortness of breath with exertion, watery eyes, chest pains, psychoses, slight to moderate thyroid enlargement, changes in blood parameters (including higher hemoglobin levels and lymphocyte counts)	22

* Occupational exposure (plating bath containing 3% NaCN and 3% copper cyanide)

Table 2. Exposure-effect relationships observed in laboratory animals exposed to HCN by inhalation.

Exposure	Species	Effects	Ref.
100 ppm, 19 minutes	Monkey	Hyperventilation, irregular heartbeat, ataxia, other effects	67
63 ppm 30 minutes	Mouse	DC_{50}, intermittent sensory irritation	58
60 ppm 30 minutes	Monkey	Somewhat elevated respiratory minute volume, changes in EEG, reduced amplitude of auditory cortical evoked potential toward end of exposure period	66
55 ppm 30 minutes	Rat	Increased transthoracic pressure, air flow and tidal volume; reduced compliance, respiratory rate and minute volume, reduced content of phospholipids in lungs	9
45 ppm 30 minutes/day 28-96 days	Dog	Breathing difficulty, vomiting, diarrhea, tremor, spasms, effects on locomotion, coma, death, histological changes in brain	4
45 ppm 30 minutes	Mouse	Animals remain lying on their sides	25
45 ppm 25 minutes	Cat	Animals remain lying on their sides	25
45 ppm 15 minutes	Dog	Animals remain lying on their sides	25
0.5 ppm 24 hours/day 4 weeks	Rabbit	No exposure-related effects on heart or lungs	42, 43

Table 3. Exposure-effect relationships observed in laboratory animals with repeated oral or subcutaneous administration of NaCN or KCN.

Exposure	Dose as CN mg/kg bw/day	Species	Effect	Ref.
NaCN, gavage 2 mg/kg bw/day, 14.5 months	1.1	Dog	Occasional convulsions, apnea, vomiting in conjunction with administration; inflammatory changes in digestive tract, degenerative changes in CNS, changes in blood profile	36
NaCN, in diet (=10.8 mg HCN/kg feed) 14 weeks	1.04	Dog	Nephrosis, hyperplasia and hypertrophy of adrenals, effects on spermatogenesis	47
NaCN, in diet (=10.8 mg HCN/kg feed) 14 weeks	1.04	Dog	Lowered T_4 in serum, somewhat higher thyroid weight, hyperplasia of thyroid, necrosis, fibrosis and atrophy of spleen, lower weight gain	48, 49
KCN, s.c. injection 1.4 mg/kg bw once a week 22 weeks	0.57*	Rat	Degenerative changes in CNS, lower vitamin B_{12} levels in liver	24, 77
NaCN, in drinking water 10 mg/liter 0.9-1 mg/kg bw/day 13 weeks	0.5	Rat	Somewhat lower weight gain (males)	34
KCN, gavage 1 mg/kg bw/day 24 weeks	0.4	Pig	Reduced T_3 and T_4 in blood, increase in blood glucose, effects on behavior	45
NaCN, gavage 0.5 mg/kg bw/day 13.5 months	0.27	Dog	Symptoms of acute poisoning after 53 weeks; inflammatory changes in digestive tract, slight degenerative changes in CNS, changes in blood profile	36
NaCN, in drinking water 3 mg/liter 0.3 mg/kg bw/day 13 weeks	0.16	Rat	No notable effects	34

*mg/kg bw/injection

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Consensus Report for Toluene Diisocyanate (TDI), Diphenylmethane Diisocyanate (MDI), Hexamethylene Diisocyanate (HDI)

May 30, 2001

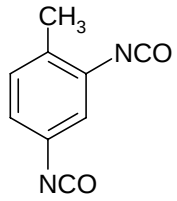
There are a large number of isocyanates, and the present document covers three of the most common ones: toluene diisocyanate (TDI), diphenylmethane-4,4´-diisocyanate (MDI), and hexamethylene diisocyanate (HDI). It also serves as an update to the Consensus Reports published in 1982 and 1988 (130, 74). Thermal breakdown products of polyurethane are not discussed.

Chemical and physical characteristics. Uses

toluene-2,4-diisocyanate (2,4-TDI)

CAS No.:	584-84-9
Synonyms:	2,4-toluene diisocyanate 2,4-diisocyanatotoluene 4-methyl-1,3-phenylene diisocyanate toluene diisocyanate

Structure:

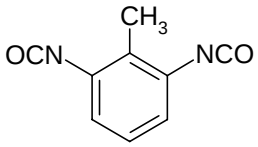


Melting point:	21 °C
Flash point:	135 °C

toluene-2,6-diisocyanate (2,6-TDI)

CAS No.:	91-08-7
Synonyms:	2,6-toluene diisocyanate 2,6-diisocyanatotoluene 2-methyl-1,3-phenylene diisocyanate toluene diisocyanate

Structure:



2,4-TDI:2,6-TDI (4:1)

CAS No.:	26471-62-5
Melting point:	12.5-13.5 °C
Flash point:	132 °C

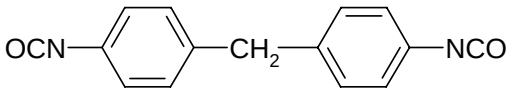
For all of the above:

Formula:	$C_9H_6N_2O_2$
Molecular weight:	174.16
Density (liquid):	1.22 g/cm^3 (25 °C)
Density (vapor):	6.0 (air = 1)
Boiling point:	251 °C
Vapor pressure:	2.7 Pa (25 °C)
Saturation concentration:	27 ppm
Conversion factors:	1 µg/m^3 = 0.138 ppb
	1 ppb = 7.239 µg/m^3

diphenylmethane-4,4´-diisocyanate (MDI)

CAS No.:	101-68-8
Synonyms:	methylenebis(phenylisocyanate)
	4,4´-diphenyl methane diisocyanate
	bis(1,4-isocyanatophenyl)methane
	4,4´-methylenediphenyl diisocyanate
	4,4´-diisocyanato-diphenyl methane
	diphenylmethane diisocyanate
	4,4´-methylphenyl diisocyanate
Formula:	$C_{15}H_{10}N_2O_2$

Structure:



Molecular weight:	250.3
Density (liquid):	1.23 g/cm^3 (25 °C)
Density (vapor):	8.5 (air = 1)
Boiling point:	314 °C
Melting point:	39.5 °C

Flash point:	196 °C
Vapor pressure:	6.7 x 10^{-4} Pa (25 °C)
Saturation concentration:	6.6 ppb
Conversion factors:	1 µg/m^3 = 0.096 ppb
	1 ppb = 10.40 µg/m^3

hexamethylene-1,6-diisocyanate (HDI)

CAS No.:	822-06-0
Synonyms:	1,6-diisocyanatohexane
	1,6-hexamethylene diisocyanate
Formula:	$C_8H_{12}N_2O_2$
Structure:	$OCN–(CH_2)_6–NCO$
Molecular weight:	168
Density (liquid):	1.04 g/cm^3
Boiling point:	212.8 °C
Melting point:	- 67 °C
Flash point:	140 °C
Vapor pressure:	1.3 Pa (20 °C)
Saturation concentration:	13 ppm
Conversion factors:	1 µg/m^3 = 0.143 ppb
	1 ppb = 6.991 µg/m^3

Isocyanates are characterized by their reactive -N=C=O group. Some diisocyanates (TDI, HDI) are volatile at room temperature and others can not be inhaled unless they are heated or in aerosol form (MDI). MDI and TDI usually occur industrially as mixtures of different isomers and/or in prepolymerized form. Industrial grade TDI is usually a mixture of 2,4-TDI and 2,6-TDI in a ratio of 4:1 or 65:35. MDI usually occurs as a mixture of several isomers and oligomers: one common mixture is approximately 30-40% diphenylmethane-4,4´-diisocyanate, 2.5-4.0% diphenylmethane-2,4´-diisocyanate, 0.1-0.2 % diphenylmethane-2,2´-diisocyanate, and the remaining 50-60% oligomers. HDI rarely occurs in monomeric form and is usually in adducts.

Isocyanates are used in the production of polyurethane, a common material for soft and hard foam plastics, insulation materials, two-component adhesives, foam rubber, and various types of paints and hardeners. TDI is used primarily in the production of low-viscosity polyurethane foam, and MDI is used for the production of harder polyurethane items (in catalyzers, for example). TDI and MDI are used in surface coatings (e.g. for chutes used in mining and agriculture), in the shoe industry for shoe soles, in the automobile industry for shock absorbers, and in a wide variety of other industries. MDI occurs in foundries in binders for casting forms, and in orthopedic surgery in casts for broken bones.

The reported odor threshold for TDI is 360-920 µg/m^3 (53). MDI is reported to be odorless (52). There is no information regarding the odor theshold for HDI.

Measuring air concentrations of TDI, MDI and HDI

Measuring isocyanates in air is complicated by several factors (97, 129). Isocyanates are extremely reactive compounds. They may occur as gases or as particles of various sizes in aerosols. The monitoring method must be specific and extremely sensitive, since the threshold values are low. Because of the high reactivity of isocyanates, samples must be taken using chemosorption, i.e. by derivitization using a reagent in solution or a reagent-impregnated solid medium, and this must be followed by chromatographic analysis. The method in general use before the introduction of chromatography was the spectrophotometric Marcali method (86). Measurements made with the Marcali method must be regarded with considerable reservation today, since the sensitivity and specificity of the method do not meet present standards. In simple exposure situations, however, the Marcali method may yield reliable values for TDI.

The reagents most widely used during the past 20 years for isocyanate derivatization in conjunction with chromatographic analysis are the following:

N-[(4-nitrophenyl)methyl]propylamine ('NITRO reagent')
9-(N-methylamino methyl)-anthracene (MAMA)
1-(2-pyridyl)-piperazine (PP)
1-(2-methoxyphenyl)-piperazine (MP)
1-(9-anthracenylmethyl)-piperazine (MAP)
tryptamine (TRYP)
di-n-butylamine (DBA)

In monitoring TDI, MDI and HDI, the most important parameters are the gas:particle ratio and the size and reactivity of any isocyanate particles that may be present. It is probable that under certain conditions (such as spraying of quick-setting MDI products) the particles react too slowly with the reagent when samples are taken on reagent-impregnated filters, and samples must therefore be taken in an impinger (4). On the other hand, particles smaller than 2 µm are not captured effectively in an impinger. It is therefore recommended that, in environments containing both quick-drying particles larger than 2 µm and particles smaller than 2 µm, sampling in an impinger be followed by sampling on a reagent-coated fiberglass filter (47, 128). In one of the methods commonly used in other countries, samples are taken in an impinger containing MP in toluene or on a fiberglass filter coated with MP (47). MAMA in toluene has been widely used in Sweden (11). For monitoring monomeric TDI, MDI and HDI in environments where these substances are generated by polyurethane production, the choice of reagent is generally not of critical importance. DBA in toluene has several advantages over other reagents (135), but disadvantages have also been found (67). As a rule, reagent-coated filters should be extracted in the field right after the sample is taken. With regard to measuring TDI, MDI and HDI in complex environments – around thermal breakdown of polyurethane materials, for example – our knowledge of the efficiency of the various reagents and sampling methods is still quite limited.

Liquid chromatography is the only method used to separate isocyanates prior to analysis. One or a combination of detectors – UV, fluorescence, electrochemical – may be used for identification and quantification (97, 129). To identify and quantify samples from complex environments it is necessary to use mass spectrometry. DBA is the reagent that has been most thoroughly investigated in conjunction with mass spectrometric analysis (54, 57, 136). International standards are being drawn up for methods based on MP, MAP, DBA, various combinations of reagents, and different sampling methods (54).

In summary, we now know that earlier measurements of isocyanates, particularly in complex environments (with thermal breakdown, for example), can be off by a wide margin – especially if they were made before the introduction of chromatography. Further, sampling in an impinger may yield measurements that are too low because the particles smaller than 2 µm were not captured. Chromatographic analysis of derivatized isocyanates without confirmation by mass spectrometry may lead to concentration estimates that are too high. When the derivatization reaction is slow, competing reactions may lead to estimates that are too low. In assessing the results of epidemiological studies, therefore, the monitoring method must always be assessed as well. If a study is to be accepted as reliable, the exposure levels must be determined by modern chromatographic methods. An exception may be made for measurements made in simple exposure situations, where determination with the Marcali method may be acceptable.

Uptake, biotransformation, excretion

In studies with rats and guinea pigs it has been shown that inhaled TDI is absorbed in the central respiratory passages and far out in the bronchioles (61). In general, isocyanates bind to proteins quite rapidly (62). Skin uptake of TDI has been demonstrated in rats after exposure 3 hours/day for 4 consecutive days (115).

Due to their –N=C=O group, isocyanates are extremely reactive substances that form adducts. Urine samples taken after exposure to isocyanates contain the corresponding amines, which can be identified if the sample is prepared by hydrolysis. Thus, toluene diamines (TDA) can be found after exposure to TDI (115), 4,4´-methylenedianiline (MDA) and N-acetyl-4,4´-methylenedianiline (AcMDA) after exposure to MDI (124), and 1,6-hexamethylene diamine (HDA) after exposure to HDI (15). It has been assumed on the evidence of *in vitro* studies that the toxicity of isocyanates is largely due to the distribution of isocyanate-glutathione conjugates to various organs via blood and the release of free isocyanate in peripheral tissues. This occurs particularly with low local concentrations of glutathione (GSH) or elevated pH (14, 102). The distribution of isocyanates in the human body, however, is largely unknown.

Data on metabolism of isocyanates are also sparse. In experiments in which rats were given 14C-TDI in diet, insoluble polymers were formed in the stomach at high doses (900 mg/kg b.w./day) in feed, but not at lower doses (60 mg/kg

b.w./day) (53). The metabolites of 2,6-TDI are excreted primarily in feces. Oral administration of 2,6-TDI in oil (900 mg/kg b.w.) resulted in formation of polymers in the digestive tract, and 2,6-TDI polymers were still in the digestive tract 72 hours later. The half time for 2,6-TDI in aqueous solution is less than 2 minutes in the ventricle and less than 30 seconds in serum (53). MDI and TDI have been found in and below the respiratory epithelium of exposed laboratory animals from the nose to the terminal bronchioles. TDI-protein complexes have been found in pulmonary tissue of guinea pigs after inhalation of TDI (60). Bronchoalveolar lavage fluid from TDI-exposed guinea pigs contained five TDI-protein complexes, of which TDI-albumin was the most prevalent (56). In humans exposed to airborne 2,4-TDI and 2,6-TDI, the substances are bound mostly to albumin in plasma (70). TDI-hemoglobin complexes have been identified in guinea pigs after inhalation of TDI (27). After rats had been exposed by inhalation to 14C-TDI, the highest concentrations of radioactivity were found in respiratory passages, digestive tract and blood, in that order. The radioactivity in plasma was linearly related to the dose of inhaled 14C-TDI, and the 14C-labeled compounds were almost entirely in the form of conjugates (63).

Biological measures of exposure

Volunteers exposed to HDI in a test chamber (3.6 ppb for 7.5 hours) had the corresponding amine (1,6-hexamethylene diamine, HDA) in hydrolyzed urine (15). The biological half time was about 1 hour. In a similar study with lower exposures (1.7 to 3.1 ppb for 2 hours), HDA levels in urine were proportional to HDI levels in air, and the half time was 2.5 hours (134). HDA could not be found in unhydrolyzed urine, which suggests that this metabolite occurs as an adduct. HDA was not detected in plasma in either of these studies (15, 134). HDA could be identified in hydrolyzed urine from car painters exposed to prepolymerized HDI (116) and production workers making HDI monomers (80). Of 22 car painters who used HDI-based paint and wore face masks with air filters, 4 had HDA in hydrolyzed urine; none of seven controls had detectable amounts (149).

Subjects exposed to 2,4-TDI and 2,6-TDI in a test chamber (5.5 ppb for 7.5 hours) had the corresponding amines in plasma and urine – identified by gas chromatography-mass spectrometry (GC-MS) after hydrolysis (125). Elimination from plasma was slow. Elimination in urine had a slow and a fast phase, the latter with a biological half time of one to two hours. Two persons were exposed to three concentrations in the range 3.4-9.7 ppb for 4 hours, and there was a correlation between TDI levels in air and TDA levels in plasma (16). Elimination from plasma had two phases. The biological half time was two to five hours for the fast phase and more than six days for the slow phase (16).

In a cross-sectional study of employees making automobile upholstery in a workplace where there was low exposure to both aerosols from spraying of MDI and HDI adhesives and thermal breakdown products from the adhesives and from TDI foam (air concentrations of MDI <0.76 ppb, HDI <0.1 ppb, TDI <0.01 ppb),

toluene-2,4-diamine (2,4-TDA) and toluene-2,6-diamine (2,6-TDA) were detected in hydrolyzed plasma from respectively 16% and 7% of the production workers, but none of the office workers (72). Some TDA isomer was found in the urine of 48% of the production workers and 21% of the office workers.

A TDI metabolite (2,6-TDA), but not TDI, could be identified in hydrolyzed urine from workers exposed to 2,6-TDI (79, 117). There was some correlation to air concentrations. Workers exposed to TDI (≤ 0.5 ppb) in a foam plastic factory were monitored for 5 weeks: the urine content of TDA fluctuated, as did the ratio between 2,4-TDA and 2,6-TDA (103). There was some correlation with air concentrations. The amounts of TDA in plasma were relatively constant, and not correlated to amounts in urine. In another study of workers in two foam plastic factories where air concentrations of TDI were 0.05-0.5 ppb and 1.4-16.6 ppb respectively, the TDA levels in plasma reflected the difference in air concentrations (69). While the workers were on vacation the levels in plasma declined with an average half time of 21 days. The levels of TDA in urine dropped also, with a half time of 5.8 to 11 days and some indication of two phases.

Workers exposed to TDI (average 4.1 ppb) in a foam plastic factory had TDA in hydrolyzed plasma and urine (135). TDA in plasma of some of the workers experimentally exposed for a short time had a biological half time of about 10 days. The amount of TDA in urine from the workers varied quite a bit, and was highest right after work. There was no clear correlation between TDI levels in air and TDA levels in plasma or urine. The levels in plasma and urine were higher and the half time in plasma longer than they were for the briefly exposed subjects in the test chamber studies (16), which lends support to the hypothesis that there is a slow compartment. In plasma from a worker in the same factory, most of the metabolite was covalently bound to albumin (70).

Methylenedianiline (MDA) could be identified in pooled samples of hydrolyzed plasma and urine from 10 workers exposed to MDI (it is unclear whether thermal breakdown was involved) (126). MDA could be identified in hydrolyzed hemoglobin from 10 of 26 workers exposed to MDI (all but three <0.3 ppb; values for the other three were 1.0, 1.8 and 2.9 ppb) (122). After alkaline extraction there were measurable amounts of acetyl-MDA (AcMDA) and lesser amounts of MDA in urine from 18 of the 26, MDA alone in 4 samples, and neither substance in 4. After acid hydrolysis the MDA levels were on average about 1/3 higher that the total of AcMDA and MDA in the previous analysis. The levels of hemoglobin adducts had no correlation to metabolites in urine (122).

In a polyurethane production facility where air concentrations of MDI were usually below the detection limit, measurable amounts of 4,4´-MDA (0.035-0.83 pmol/ml) and AcMDA (0.13-7.61 pmol/ml) could be found in urine in 15 of 20 workers after alkaline extraction, and MDA values were 6.5 times higher after acid hydrolysis. MDA was found as hemoglobin adducts in all the examined workers, and one worker also had adducts of AcMDA. Plasma levels of 4,4´-MDA ranged from 0.25 to 5.4 pmol/ml, up to 120 fmol/mg of which was covalently bound to albumin (124).

2,4-TDA, 2,6-TDA and 4,4´-MDA could be found in hydrolyzed urine from 15 workers at a workplace where TDI- and MDI-based polyurethane was heated (26). The levels fluctuated widely from day to day. Levels of these metabolites in plasma were much more stable. In four of the monitored workers the levels of MDA declined during an exposure-free period, with biological half times of 2.5-3.4 days in urine and 10-22 days in plasma.

It has long been known that some persons exposed to isocyanates form antibodies specific to conjugates of the isocyanate and serum albumin (40, 147). These are of doubtful pathogenic relevance, but may be used as biomarkers of exposure (for those persons who develop antibodies). Three percent of workers exposed to spray aerosols of glue based on MDI or HDI had specific IgE antibodies, while 33% had IgG specific to MDI, 32% to TDI and 12% to HDI (72).

After exposure stops, the titer of specific antibodies declines (22, 81), but it may remain elevated for as long as five years (71).

To sum up, it seems that in principle conditions exist for biological monitoring of exposure to isocyanates. With regard to biomarkers for exposure, it is possible to analyze metabolites in plasma and urine, although it involves considerable work with sample treatment, determination by chromatography- mass spectrometry, and quality control. Levels of metabolites in urine samples taken soon after an exposure following a period without exposure reflect the exposure of the previous hours, whereas levels in plasma reflect more long-term exposure.

However, there is much that is not known – both generally and about the individual monomers. In all cases the relative importance of gas and particles in exposures is far from clear. The relevance of skin uptake is still largely unstudied. A special problem with biomonitoring is the difficulty of differentiating exposure to a diisocyanate from exposure to its amines and aminoisocyanates. For HDI the analytical problems are such that only high exposures can be detected.

Specific IgG in serum increases with exposure to HDI, TDI and MDI, but in only some exposed persons. The temporal relationship to exposure is not clear. The concentrations persist for months and even years after exposure has stopped. Virtually nothing is known about quantitative relationships between air concentrations and specific IgG. Similarly, very little is known about the relation between antibody concentration and risk of health problems. The only existing data pertain to respiratory symptoms and exposure to HDI, TDI and MDI in environments with thermal breakdown. Specific IgE is probably of very limited value for estimating exposure and risk.

Toxic effects

Animal data

For rats, the calculated LD50 for a single oral dose of TDI is 5.8 g/kg (152). The LC50 for exposure via inhaled air, 4 hours/day for 2 weeks, was 9.7 ppm for mice, 12.4 ppm for guinea pigs, and 13.8 ppm for rats. Watery eyes, salivation,

agitation and hyperactivity were noted in the animals during the exposures (31). Inhalation of up to 18 ppb 2,4-TDI for 3 hours caused no change in the respiratory rate of mice, either after a single exposure or after the exposure was repeated for several days. Inhalation of 23 ppb for 3 hours did reduce the respiratory rate, however, and the effect was enhanced when the exposure was repeated the following day (120). Acute inhalation exposure to high concentrations of TDI causes extensive damage and necrosis in pulmonary epithelium, and leads to death of the animals by occlusion of bronchioles with necrotic tissue, edema in mucous membranes, and the severe inflammatory reaction (31). Mice that inhaled TDI (time-weighted average 400 ppb) 6 hours/day for 5 days had squamous metaplasias, exfoliative changes, erosion and ulceration in nasal epithelium (18). Exposure to 98 ppb TDI 6 hours/day for 4 days caused inflammation and necrosis in respiratory epithelium of mice (51). MDI and TDI present about the same toxic picture in experimental animals. In an inhalation toxicity study (113), the acute (4 hours) LC50 for rats exposed to an aerosol mixture of respirable MDI monomers and polymers (with ≤ 0.005% w/w phenyl isocyanate) was 490 mg/m3 (95% confidence interval 376-638 mg/m3). Two weeks of exposure to a mixture of MDI polymers with 44.8-50.2% monomer at a concentration of 13.6 mg/m3 resulted in severely retarded growth and some deaths, and 13 weeks of exposure to 12.3 mg/m3 also caused elevated mortality and retarded growth (113).

For mice, inhalation of 50 or 150 ppb TDI (2,4-TDI:2,6-TDI, 4:1) 6 hours/day for 104 weeks resulted in significantly lower body weights in the high-dose group and elevated mortality among females in both exposure groups (survival in controls 40%, low-dose group 23%, high-dose group 26%). No such increase in mortality was seen for the males (49). Interstitial pneumonitis and necrotic changes in nasal mucosa were observed at both exposure levels (49). Rats were exposed to 50 or 150 ppb airborne TDI 6 hours/day, 5 days/week for 108 weeks (females) or 110 weeks (males): they initially lost weight, but weight development was normal after 12 weeks of exposure. There were no effects on survival and no observed changes in mucous membranes in the upper respiratory passages (49).

In rabbits and guinea pigs, toluene diisocyanate induces bronchial hyper-reactivity to acetylcholine, with a dose-response relationship (37, 38, 89, 120). Increased bronchial response to acetylcholine was observed in guinea pigs after 4 x 1 hours of exposure to TDI (unspecified isomer) in concentrations of 10 or 30 ppb, but not 5 ppb (90). In some experiments the animals were pre-treated with capsaicin, and it was found that this counteracted the isocyanate-induced increase in bronchial reactivity. This probably indicates that neuropeptides are involved in the pathophysiological sequence (90, 109). Guinea pigs developed respiratory tract hypersensitivity to TDI after dermal exposure (59).

Wistar rats were exposed to an MDI aerosol (a mixture of polymers with 44.8-50.2% monomer) 6 hours/day, 5 days/week for up to two years: exposure to 576 ppb resulted in hyperplasia of basal cells in olfactory epithelium and

accumulation of alveolar macrophages with surrounding fibroses in the lungs (112).

Bronchial reactivity in guinea pigs was increased more by dermal application of MDI (intradermal 0.0003-0.3%; epidermal 10-100%) than by inhalation exposure (2775 or 3390 ppb) (110). Bronchoalveolar lavage fluid from guinea pigs with TDI-induced bronchial hyperreactivity contained elevated numbers of eosinophilic granular leukocytes (eosinophils) and elevated concentrations of cysteinyl leukotrienes, leukotriene B4 (LTB4) and prostaglandin F2_ (PGF2_) (111). Several polyisocyanate prepolymers were assessed for their potential to induce contact allergy (delayed dermal hypersensitivity) in a study with guinea pigs, using the method described by Buehler (154). TDI and HDI were among the substances tested, and both were strongly allergenic. Eighteen of 20 animals were sensitized to TDI, which was ranked as a grade V allergen on the Magnusson-Kligman scale (78); the induction concentration was 5% and the test concentration was 1%. Fourteen of 20 animals were sensitized to HDI, which was ranked as a grade IV allergen on the Magnusson-Kligman scale (78); the induction concentration was 1% and the test concentration was 0.1%. The control animals tested negative. Isocyanates have also been tested on mice for skin sensitization, measured as ear swelling. TDI (131, 133, 137), MDI (131, 133) and HDI (133) were found to be contact allergens. HDI was observed to be more potent than MDI, which in turn was more potent than TDI (133). The SD50 (the dose that sensitized 50% of animals) was 0.088 mg/kg for HDI, 0.73 mg/kg for MDI and 5.3 mg/kg for TDI. It was also demonstrated in this study that the different isocyanates cross-reacted and that TDI, which was the least potent sensitizing agent, also had the lowest tendency to cross-react (133). A 5% (but not 1%) solution of TDI (2,4-TDI:2,6-TDI, 4:1) caused ear swelling in previously unexposed mice. After sensitization, the 1% solution also caused ear swelling (131). Sensitization was observed in 7 of 8 guinea pigs seven days after dermal application of TDI (2,4-TDI:2,6-TDI, 4:1) (59). A number of other studies with guinea pigs, which were made by methods other than those prescribed in the OECD Guideline (99), have also shown that MDI and TDI are medium-strong to strong contact allergens (59, 66, 110).

Human data

Irritation of respiratory passages

Vid TDI-koncentrationer (exponeringen vanligtvis ej närmare karakteriserad men Seven men exposed to TDI (the substance is usually not more closely specified in these studies, but in most cases is probably a 4:1 mixture of 2,4-TDI and 2,6-TDI) concentrations exceeding 100 ppb (724 _g/m3) immediately showed symptoms of respiratory irritation (cough, nasal congestion, throat irritation) (91). It has long been known that exposure to TDI in concentrations exceeding 500 ppb results in irritation of the nose and throat (152). In a study of 379 TDI-exposed workers at 14 workplaces, 30% (n = 115) had symptoms that could with some confidence be

attributed to their exposure (32). All 12 persons exposed to (unspecified) isocyanates in the concentration range 30-70 ppb had symptoms in the form of cough, dyspnea and/or irritation of mucous membranes in nose and throat (43). In this study, at exposures below 30 ppb no immediate symptoms of respiratory irritation were seen in persons who were not hypersensitive to isocyanate (43). A WHO report published in 1987 states that exposure to TDI concentrations exceeding 50 ppb causes irritation of eyes and upper and lower respiratory passages (53). This report does not mention the source of the original data. The exposure measurements in most of the studies reviewed here were made by methods that do not meet present standards.

Exposure to MDI is irritating to skin, eyes and respiratory passages (52). For MDI, the relationship between symptoms and exposure levels is still insufficiently known. In one study, nose and throat irritation was observed in about half of 13 employees who had been transferred because of their reactions to isocyanate exposure and in a few of the 20 who had stayed in the same jobs and were still being exposed. Precise exposure levels are not given in this study, but it is reported that the exposure limit for MDI (96 ppb) was reached during some operations and that higher peaks may have occurred (65).

Asthma and asthma-like symptoms

People who become hypersensitive to isocyanates develop symptoms such as coughing, rales and dyspnea at exposures below 20 ppb (10, 13, 98, 151). Isocyanate-induced asthma often begins with coughing and breathing difficulty in conjunction with exposure, but sometimes bronchial obstruction with exertion or on exposure to other bronchoconstricting stimuli is the only symptom of incipient isocyanate asthma. The asthmatic reaction may be of either the immediate (within 30 minutes of exposure) or delayed type (3 to 6 hours after exposure), and may also be biphasic. Persons with isocyanate asthma sometimes have rhinitis and/or conjunctivitis, and may have urticarial reactions as well (8).

In a prospective cohort study, 89 previously unexposed workers who were employed in the manufacture of TDI were followed for 2.5 years (20). TDI levels registered by stationary monitors (8-hour time-weighted averages, 10 occasions) were in the range 3-54 ppb, median 6.5 ppb; personal monitors showed 1-25 ppb, median 5 ppb. The TDI-exposed workers had a significantly higher occurrence of symptoms involving the lower respiratory passages (cough, wheezing, chest tightness, dyspnea etc.) than unexposed controls (20).

A review article by Musk *et al*. addresses the problems of defining a relationship between isocyanate exposure and effects on health and determining the relative importance of brief exposures to high concentrations and continuous exposure to low concentrations (96). It has been proposed that, for healthy persons, brief episodes of high exposure are more likely to lead to isocyanate asthma than long-term exposure to lower concentrations. The relative importance of high, short-term exposures and low, long-term exposures in the development of isocyanate asthma is still unclear, however.

White *et al*. (148) examined 203 women who were occupationally exposed to TDI while sewing automobile seat covers of polyurethane plastic. In some parts of the factory there was exposure to both TDI and fibrous dust. In an initial sub-study of 68 women who worked in the factory, 48 of whom worked with the polyurethane material, 17 (25%) were found to have respiratory symptoms. Ten of these had developed symptoms and three had become worse after they began work (and thus exposure) at the factory. It is not clear from the report how many of the 48 upholstery workers reported periods of breathing difficulty and wheezing on the questionnaire the subjects filled in for a medical interview. Another sub-study reports periods of dyspnea and wheezing in 30% of the women: 24% worked with the upholstery material and 11% had other jobs where they were not exposed to TDI. The difference between these groups was not statistically significant, however. Furthermore, the group exposed to isocyanates contained more smokers (55%; 42% in controls), and there was no attempt to adjust for this difference. The levels of TDI were monitored in the breathing zone of the women while they were working, and also near the needles of the sewing machines and the scissors used to cut the plastic. Air samples were collected for 5 to 29 minutes (= 5 to 29 liters of air) and analyzed with HPLC. One shortcoming of this study is that it is not clear how many measurements were used to calculate the exposure levels. The measured air concentrations of TDI were between 0.3 and 3 ppb, and in the opinion of the authors there were no exposure peaks that deviated significantly from these values. Dust concentrations reportedly did not exceed 1 mg/l (1000 mg/m3) (148). The most serious shortcomings of this study are inadequately reported exposure data and flawed data analysis.

In an English study (93), exposure conditions for 27 workers whose isocyanate asthma had been reported to a register were compared with conditions for 51 persons in the same factory who did not have asthma. Individual exposures to TDI were measured with the paper tape method (described in Reference 29) during the entire workshift, and reported as 8-hour time-weighted averages (TWA) and exposure peaks. The 8-hour TWA was somewhat higher for the asthma cases (1.5 ppb; 95% confidence interval 1.2-1.8 ppb) than for controls (1.2 ppb; 95% confidence interval 1.0-1.4 ppb). Individual top exposures were between 1 and 50 ppb, the same for cases and controls. For persons whose exposure was higher than the median value for the control group (1.125 ppb), the odds ratio for developing asthma was 3.2 (95% confidence interval 0.96-10.6). An exposure increase of 0.1 ppb corresponded to an 8% increase in risk of developing asthma. Time from hiring to the onset of asthma symptoms ranged from less than 1 month to 23 years (median 21 months). The authors describe the study as a case-referent study, but it is a dubious description since the cases are defined by both their illness and their exposure. Exposure conditions for the asthma cases and for controls were estimated later by monitoring workers doing similar jobs. A prerequisite for the study to be valid is that workers with low and high exposure within in the same area had the same chance to be diagnosed as having asthma. The authors do not make clear whether this requirement was met. The study, for

this reason and others, provides no solid basis for any conclusions on dose-response or dose-effect relationships.

In the cohort study reviewed above (20), a few of the workers hypersensitive to TDI developed severe bronchial obstruction after bronchial provocation with 5 ppb TDI. Bronchial provocation with different concentrations of isocyanates elicited asthma-like reactions in workers who had previously experienced coughing and chest tightness in conjunction with exposure to isocyanates (6), see Table 1. In brief, concentrations of ≤ 20 ppb triggered asthma symptoms in 16 of 59 workers exposed to MDI, 12 of 40 exposed to TDI and 3 of 42 exposed to HDI (6). Bronchial obstruction was observed in four workers hypersensitive to isocyanates at TDI exposures estimated by the authors of one study to be about 1 ppb, though it is not clear how this exposure level was determined (24). In another study, specific bronchoprovocation tests were given to persons with suspected occupational asthma and exposure to TDI, MDI or HDI at work (average exposure time 8.8 years). They were exposed in a test chamber to the individual isocyanates in concentrations of 5 to 20 ppb for up to 2 hours. Four of six subjects had positive reactions to TDI, 10 of 17 to MDI, and 15 of 39 to HDI (25). For persons who have developed TDI asthma, it seems to be the cumulative dose that determines whether symptoms appear. In a study by Vandenplas *et al*. 4 persons with isocyanate asthma were each exposed to TDI on 3 or 4 occasions. The total dose on each occasion was equivalent to the dose that had previously been shown to cause a 20% reduction of FEV_1 for that subject, but the concentration ranged from 5 to 20 ppb and the exposure time ranged from 1 to 90 minutes. It was found in this study that exposure to low concentrations of TDI for longer periods triggered the same reaction as exposure to higher concentrations for shorter periods, provided that the total dose was the same (140).

Table 1. Results of experimental provocation exposure of 141 workers who were occupationally exposed to isocyanates and had work-related dyspnea. Subjects were exposed to 5 ppb for 15 minutes, followed by 10 ppb for 30 minutes, followed by 20 ppb for 5 minutes. The table shows, for each tested diisocyanate, the number of persons having asthma-like reactions at each concentration (6).

	5 ppb	10 ppb	20 ppb
MDI (n=59)	6	2	8
TDI (n=40)	1	3	8
HDI (n=42)	0	2	1

Six cases of occupational asthma were identified in a group of 48 spray painters who were exposed to TDI, MDI and HDI at work. Exposure levels were not determined in this study (123). Foundry workers who had developed asthma with hypersensitivity to isocyanates had positive reactions to one hour of exposure to MDI (bronchial provocation in a test chamber). The exposure levels in this study were on average 12 ppb and never higher than 20 ppb (151).

Bronchial inflammation due to isocyanate asthma, regardless of which isocyanate is the cause, resembles that observed with other types of asthma. Bronchial biopsies from patients with isocyanate asthma have elevated levels of activated eosinophils in mucosa and submucosa and higher numbers of mast cells in epithelium (35). Bronchoalveolar lavage fluid and biopsies of respiratory mucosa from persons with isocyanate asthma contain elevated numbers of eosinophils and activated lymphocytes (83, 84, 119). Induced sputum from patients with isocyanate asthma contains elevated numbers of eosinophils (76). Indications of eosinophil activation (eosinophil cationic protein (ECP)) in blood increase after provocation with isocyanate (85). In patients with isocyanate asthma, acute exposure to TDI (unspecified isomer) leads to a temporary increase in the number of lymphocytes containing interleukin 4 in respiratory mucosa, possibly indicating a preponderance of Th2 lymphocytes (77). Early in the reaction to TDI there is a migration of neutrophilic granular leukocytes (neutrophils) to the respiratory passages. This has been observed with TDI provocation in persons occupationally exposed to TDI (34) and also in animal studies (46, 111). It resembles the early phase of the allergic asthma reaction, when an invasion of neutrophils can be observed in airways (28, 30, 94). Asthmatics with delayed reactions triggered by TDI (unspecified isomer) also have elevated numbers of eosinophils and CD8-positive lymphocytes in blood (36). Stimulating mononuclear blood cells with antigens to TDI-, MDI- and HDI-albumin complexes liberates more histamine releasing factors in patients with isocyanate asthma than in asymptomatic controls exposed to diisocyanate (45, 73). The authors suggest that liberation of histamine releasing factors on specific provocation might serve as a biological marker for isocyanate asthma.

Some studies report a correlation between certain HLA types and risk of developing TDI-induced asthma. There was an elevated risk of developing asthma as a result of TDI exposure for persons who had the allele DQB1*0503 or the allele combination DQB1*0201-0301, whereas the risk was reduced for persons with the allele pair DQB1*501 or the combination DQA1*0101-DQB1*0501 (12). Other researchers, however, have not been able to confirm that special HLA class II alleles are relevant in this context (114) and no definite conclusions can yet be drawn regarding a relationship between HLA type and risk of isocyanate asthma.

In a retrospective study (17-year follow-up) of 300 exposed workers, a correlation was found between hypersensitivity to TDI and exposure to high concentrations of TDI (above 50 ppb, usually a 4:1 mixture of 2,4-TDI and 2,6-TDI) (108). The exposure was brought down below 20 ppb, and no new cases of

TDI hypersensitivity were subsequently seen in a 3-year retrospective follow-up of workers who worked with TDI daily (108). In another study, IgE antibodies specific to isocyanates – TDI (unspecified isomer), MDI, HDI – were found in 20 of 94 exposed workers. There was no significant correlation between specific and total IgE (92). This study includes no analysis of a relationship between symptoms and the presence of IgE antibodies. In various other studies, 10 to 30% of those with isocyanate asthma have been found to have circulating IgE antibodies specific to albumin-bound isocyanate and/or positive reactions to isocyanates in prick tests. (21, 25, 58, 64, 143, 151). Despite the fact that the isocyanates are quite dissimilar in chemical structure, cross-reactions have often been observed both *in vitro* (IgE, IgG) and in tests of specific bronchial reactivity (5, 25, 132). This is believed to be due to the high reactivity of isocyanates and their rapid formation of complexes with more high-molecular substances, with resulting sensitization to these new complexes rather than to the isocyanate itself (39). The correlation between the presence of isocyanate-specific IgE antibodies in blood and positive reactions to specific bronchial provocation (25) or respiratory symptoms (9) is weak, which may indicate that isocyanate-specific IgE antibodies have only a minor role in isocyanate asthma. In most persons who have isocyanate asthma it is impossible to find circulating IgE antibodies specific to isocyanate.

In car painters exposed to vapors and aerosols containing HDI (prepolymers and monomer), titers of IgG specific to HDI prepolymers (but not the monomer) were significantly higher than in unexposed controls (147). No HDI-specific IgE antibodies were found in this study.

For many people, the asthma persists despite breaking off the exposure, with both symptoms and elevated bronchial reactivity to direct stimuli (82, 101). It is important to diagnose isocyanate asthma early and stop the exposure of persons who develop it, since early intervention will alleviate the asthma symptoms and may eliminate the asthma completely (107).

Alveolitis

There are a few reported cases of alveolitis caused by exposure to airborne TDI, MDI and HDI. This alveolitis is characterized by restrictive reduction in lung function, interstitial fibrosis, increase of CD8-positive cells in bronchoalveolar lavage fluid (CD4/CD8 < 1.0) and IgG antibodies specific to albumin-bound isocyanate (7, 139, 150, 153). In a total of 1,780 isocyanate-exposed workers, Baur (7) identified 14 cases of dyspnea and fever associated with exposure to isocyanates. These persons had signs of alveolitis on lung x-rays and/or restrictive reduction in lung function and/or reduced diffusion capacity and/or IgG antibodies against albumin-bound TDI, MDI or HDI in serum. Bronchoalveolar lavage and biopsies from the respiratory tract showed inflammatory changes, but no isocyanate-specific IgE antibodies were found in serum. The average exposure time was 6 years (0.5-20 years) but cumulative exposures were neither calculated nor estimated. Baur (7) found the occurrence of alveolitis to be about 1%, whereas Vandenplas *et al*. (141) found a prevalence of 4.7% among workers

occupationally exposed to resins containing MDI or MDI prepolymers. In both these studies it was remarked that exposure to MDI was more commonly associated with isocyanate-induced alveolitis than exposure to TDI or HDI. Most of the alveolitis cases in the Vandenplas study (141) had symptoms so severe that they had been forced to quit their jobs soon after the symptoms appeared, leading the authors to postulate that the 'healthy worker' effect for isocyanate-induced alveolitis may be quite large, and that alveolitis caused by isocyanate exposure is probably more common than these studies indicate. Isocyanate-induced alveolitis seems to affect non-smokers more than smokers (7).

Other effects on lung function

Long-term exposure to TDI in concentrations below 20 ppb usually causes no acute symptoms, but it has been argued that it may lead to reduced lung function (105, 144, 145). A cross-sectional study of workers exposed to MDI (usually below 20 ppb; a few measurements showing concentrations up to 87 ppb) revealed that their lung function was lower than that of an unexposed control group (106). Workers (n = 65) occupationally exposed to air concentrations of HDI too low to cause symptoms (below the detection limit in 92% of measurements), as well as various organic solvents, showed a small but statistically significant decline of lung function (FEV_1, FVC) in comparisons with unexposed controls (n = 68) and workers occupationally exposed to organic solvents alone (n = 40). This 2.5-year prospective study, however, does not include a group exposed to HDI alone (2).

The above findings are contradicted by the results of several other studies. In a 9-year study of asymptomatic workers exposed to TDI, their decline in lung function was no different from that in an unexposed control group (1). TDI-exposed workers with symptoms, however, showed a more rapid decline in lung function than the unexposed controls (1). A study by Butcher *et al*. (20) revealed no effect on lung function after 2 years of exposure to TDI in the concentration range 3-54 ppb (average values for 8-hour samples). In a study by Musk *et al*. (95), 107 workers in a factory producing polyurethane foam were followed for 5 years. During this period a total of 2,573 monitoring measurements (20 to 60 minutes) of TDI and MDI were made in the breathing zone of the workers. The average TDI concentration was 1.2 ppb, and 90% of the measurements were below 5 ppb. The average MDI concentration was 0.6 ppb, with 90% of measurements below 2.2 ppb. No information on exposure peaks is given. Changes in lung function during the 5 years of the study were the same for exposed individuals as for controls. No increase in respiratory symptoms and no decline in lung function were found in the exposed group (95). In a 4-year follow-up of TDI-exposed workers, lung function changes in 57 exposed workers were the same as those in 24 workers in a control group. When the exposed workers were divided into three exposure groups, however, it was found that the 15 workers in the high-exposure group (TWA 8.2 ppb, with individual top exposures briefly exceeding 30 ppb) had a more rapid decline in lung function (measured as

average mid-expiratory flow, FEV_1/FVC, and end-expiratory flow) during the period than the 14 workers in the medium-exposure group (average TDI exposure 1.7 ppb, individual top exposures 3-14 ppb), the 28 workers in the low-exposure group (average 0.1 ppb, top exposures below 1 ppb), and the control group (100).

In a study by Hathaway *et al*., no effect on lung function was found in 43 workers after 6 years of occupational exposure to HDI, HDI biuret and HDI trimer (HDI adducts), when they were compared with 42 controls. HDI concentrations in this study were about 0.5 ppb (2 hours of exposure), and the calculated 12-hour TWA was 0.1 ppb. Daily top exposures averaged 2.9 ppb (44).

Tornling *et al*. found no difference in lung function changes in a 6-year follow-up study of non-smoking car painters exposed to HDI and HDI biurettrimer when they were compared with non-smoking controls who were repair shop metal-workers and mechanics (138). Exposed smokers, however, had a greater annual loss of lung function (FVC, VC, FEV_1) than smokers in the control group. The meaning of this is difficult to interpret, since total tobacco exposure is not taken into account – the only information is whether the subject was a smoker, ex-smoker or non-smoker. Average exposure in this study was 0.2 ppb, but concentrations exceeding 286 ppb were not rare. A significant correlation was found between decline in FEV over time and the number of occasions with high peak exposure. This may possibly indicate that, for smokers, decline of lung function resulting from HDI exposure depends more on brief episodes of high exposure than on the average long-term exposure. The number of non-smokers in this study was too small to support any general conclusions drawn from observed effects in this group. Further, there may have been some exposure of the controls (especially the metalworkers).

Pham *et al*. examined 318 workers: 83 not exposed to isocyanates, 117 indirectly exposed and 118 directly exposed to MDI (106). The concentration of airborne MDI was on most occasions below 20 ppb, but there were a few exposure peaks up to 87 ppb. The exposed groups had a slight restrictive reduction in ventilation capacity when compared with unexposed controls.

Effects on skin

In several case reports of isocyanate-exposed workers with allergic contact eczema on hands, arms and face, contact allergy (delayed dermal hypersensitivity) to MDI, TDI and/or HDI has been diagnosed by patch tests (17, 23, 33). Of 15 workers exposed to TDI in the range 70-170 ppb, 5 had a positive patch test for TDI and 3 had contact eczema caused by TDI (48). Rothe described 12 cases of contact allergy and allergic contact eczema caused by occupational exposure to MDI (118). In some cases patients have positive test reactions to several isocyanates, and occasionally to methylene dianiline (MDA) as well – often despite the fact that they had not previously been exposed to the particular substance that induced the reaction (33, 68). This has been interpreted as evidence of cross-reactivity. The patients had been sensitized in jobs such as mold sprayer, car painter, spray painter and medical technician. Several of them had become

sensitized because of inadequate protection against skin exposure (work with worn-out gloves or no gloves, for example), although working conditions were otherwise good (33, 68). In several cases sensitization occurred after a few weeks or months of exposure at work (33). In some reported cases the patient had MDI- or HDI-induced asthma in addition to the contact eczema (33).

Teratogenicity, mutagenicity, carcinogenicity

Animal data, in vitro studies
TDI (2,4-TDI:2,6-TDI, 4:1) and MDI, but not HDI, were mutagenic in *Salmonella typhimurium* strains TA1538, TA98 and TA100 with metabolic activation (3). Changes in DNA conformation were observed in DNA from calf thymus after *in vitro* exposure to TDI (2,4-TDI:2,6-TDI, 4:1), but no such effect was obtained with exposure to MDI or HDI (104). Diisocyanates and their metabolites (including diamines) form adducts with DNA (142). *In vitro* incubation with TDI (2,4-TDI:2,6-TDI, 4:1) caused double strand breaks in leukocytes (88), although *in vitro* exposure to pure TDI caused no DNA damage. It was concluded that the damage is caused by metabolites. On contact with water isocyanates form aromatic amines, many of which are carcinogenic. TDI (2,4-TDI:2,6-TDI, 4:1) and a mixture of 45% MDI, 25% 4,4´-methylenediphenyl triisocyanate and 30% unspecified agent caused chromosome aberrations in human lymphocytes after 24 hours of incubation, without metabolic activation (75).

TDI (86% 2,4-TDI, 14% 2,6-TDI) given orally to mice 5 days/week for 105 weeks (120 or 240 mg/kg/day to males, 60 or 120 mg/kg/day to females) increased the occurrence (significant dose-response trend) of hemangiomas, hemangiosarcomas and hepatic adenomas in the females, but not in the males (49). Oral doses of the same substance given to rats 5 days/week for 106 weeks (30 or 60 mg/kg/day to males, 60 or 120 mg/kg/day to females) led to significant increases in the occurrence of subcutaneous fibromas and fibrosarcomas in the males. The males in the high-dose group also had significantly more pancreatic adenomas than controls ($p = 0.034$) (49).

Long-term inhalation exposure to 50 or 150 ppb TDI (2,4-TDI:2,6-TDI, 4:1) 6 hours/day did not increase the occurrence of tumors in either mice (104 weeks of exposure) or rats (108-110 weeks of exposure) (49).

In a long-term toxicity and carcinogenicity study with Wistar rats, inhalation of 19, 96 or 576 ppb MDI (a mixture of polymers with 44.8-50.2% MDI monomer), 6 hours/day, 5 days/week for 2 years, resulted in lung adenomas in 10% of the males and 3% of the females in the highest exposure group. One of 60 males in the high-exposure group also had a lung adenocarcinoma. No lung tumors were found in controls. In this study, two years of exposure to 576 ppb MDI was associated with an elevated occurrence of lung tumors, whereas concentrations of 96 ppb or less did not increase the frequency of tumors (112).

2,4-TDA given to mice in feed (100 or 200 mg/kg feed) for 101 weeks increased the occurrence of hepatocellular carcinomas and lymphomas in the

females (49). 2,4-TDA given to rats in feed (0.1%) for 36 weeks resulted in liver carcinomas (55). Rats were given (ad libitum) 2,4-TDA in diet, 125 or 250 mg/kg feed for 40 weeks followed by 50 or 100 mg/kg feed for 63 weeks. An elevated occurrence of hepatocellular cancers was seen in both sexes (49). No such effects were seen with oral administration of 2,6-diaminotoluene. 2,6-TDA in feed given to mice (50 or 100 mg/kg feed) and rats (250 or 500 mg/kg feed) for 103 weeks did not increase the frequency of tumors (49).

MDA (4,4´-methylenedianiline) was tested for carcinogenicity in an NTP study (146). There was a dose-dependent increase of hepatocellular nodules in exposed rats. Mice developed hepatocellular cancer. Thyroid adenomas and carcinomas were seen in the highest dose groups in both species. Smaller or poorly documented studies also indicate a carcinogenic effect. According to the IARC, there is 'sufficient evidence' that MDA is carcinogenic to experimental animals (50).

According to the IARC, there is 'sufficient evidence' that TDI is carcinogenic to experimental animals (51), and 'limited evidence' that MDI is carcinogenic to experimental animals (52).

Wistar rats were exposed to MDI 6 hours/day on days 6 to 15 of gestation. A slight increase in the occurrence of asymmetrical sternums was observed in fetuses at 864 ppb, but not at 288 ppb, and no other anomalies were noted in fetuses (19). Food intake by the mothers dropped during the exposures, but no indications of toxicity were observed and their weight development was no different from that of controls (19).

Human data

Inhalation of a mixture of MDI (60%), various triisocyanates (30%) and undefined isocyanates (10%) in concentrations of 5 to 20 ppb increased the occurrence of double strand breaks in the leukocytes of a 51-year-old worker occupationally exposed to MDI and MDI oligomers (87). Analyses were made both before and two hours after an exposure, after the subject had been away from work for 5 days. As a further control, the blood of a healthy unexposed person was also examined (87).

Epidemiological studies have revealed no increase in cancer risk for people exposed to isocyanates. Hagmar *et al*. made a study of cancer incidence among 4,145 employees who were occupationally exposed to TDI (unspecified isomer) and MDI at nine polyurethane production facilities. No increase in cancer incidence could be related to the exposure (42). No increase in the occurrence of malignant tumors was found in a case-referent study of 7,023 employees at these workplaces (an expansion of the group covered in Reference 42). In this study exposure levels were 3.6-414 ppb for TDI and less than 1 ppb for MDI, and exposures ranged from a few days to more than 10 years (41). Sorahan *et al*., in a cohort study of 8,288 workers at 11 polyurethane production facilities in the U.K., found no increase in occurrence of malignant tumors (127). In this study the exposure time was at least 6 months and the exposures were estimated from

historic data on the jobs done and the associated exposure level. A slight elevation in the occurrence of pancreatic and lung cancers could be observed among the women, and was interpreted by the authors as an effect of smoking, possibly in combination with the occupational exposure (127). No increase in the incidence of malignant tumors was seen in a cohort study of 4,611 employees at four different polyurethane production plants in the U.S.A. In this study, estimated exposure to TDI was below 5.5 ppb during the later part of the observation period but had previously been higher. The studied cohort had a low average age, and average duration of employment was only 2.4 years – circumstances which, in the expressed opinion of the authors, make the results of the study inconclusive (121).

In its updated summary evaluations of carcinogenic risks to humans, the IARC has placed TDI in Group 2B: 'possibly carcinogenic to humans,' and MDI in Group 3: 'not classifiable with regard to carcinogenicity to humans' (51, 52). For TDI, MDI and HDI, no studies were found of toxic effects on human reproduction or embryotoxic effects on humans (51, 52)

Dose-response / dose-effect relationships

More pronounced bronchial reactivity to acetylcholine was noted in guinea pigs after repeated exposure (4 x 1 hour) to TDI in concentrations of 10 or 30 ppb, but not 5 ppb. Mice exposed by inhalation to 23 ppb 2,4-TDI for 3 hours had a slower respiratory rate, and the effect was enhanced when exposure was repeated the following day.

In the studies in which irritation of eyes and respiratory passages was examined, persons hypersensitive to isocyanates could not be differentiated from those with normal sensitivity. It was also impossible to differentiate the effects of irritation, often accompanied by a cough, from the asthma symptoms of those who had isocyanate asthma.

Asthma-like symptoms (periods of dyspnea and wheezing) were observed in a rather poorly controlled study in which it was concluded that 2.5 years of occupational exposure to 0.3-3 ppb TDI increased the risk of developing such symptoms. This study is flawed by inadequate data presentation and data analysis. In another study with higher exposures, it was found that long-term exposure to TDI concentrations in the range 1-25 ppb (median 5 ppb) may lead to asthma symptoms in previously healthy persons. Persons hypersensitive to isocyanates may develop asthma symptoms on provocation with a TDI concentration estimated at 1 ppb.

Too little is known of the relationship between exposure levels and the development of alveolitis. Six years of occupational exposure to HDI (TWA 0.5 ppb, daily top exposures on average 2.9 ppb) had no effect on lung function changes, which were the same in exposed workers and controls.

Several studies were excluded from this description of dose-response relationships because they contain exposure measurements made with methods that do not meet present standards.

Conclusion

The critical effect of exposure to diisocyanates is development of asthma. Isocyanate asthma has been observed in persons occupationally exposed to TDI at workplaces where air concentrations ranged from 1 to 25 ppb (median 5 ppb). In one study of substandard quality, the development of asthma-like symptoms such as dyspnea and wheezing was correlated to exposure levels of 0.3 to 3 ppb TDI.

Asthma symptoms have been observed in individuals hypersensitive to isocyanates in conjunction with TDI levels of 5 ppb and estimated TDI levels of 1 ppb.

TDI, MDI and HDI have been shown to be sensitizing to skin.

TDI and a mixture of MDI and its polymers have been shown to induce cancer in experimental animals. Both substances are considered genotoxic. For HDI, there are no data on cancer or genotoxicity.

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Summary

Montelius J (ed). *Scientific Basis for Swedish Occupational Standards.* XXII. Arbete och Hälsa 2001:20, pp 1-96. National Institute for Working Life, Solna.

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, 2000 through June, 2001.

Key Words: Diphenylmethane diisocyanate (MDI), Ethylenethiourea, Hexamethylene diisocyanate (HDI), Hydrogen cyanide, α-Methylstyrene, Occupational exposure limit (OEL), Potassium cyanide, Risk assessment, Scientific basis, Sodium cyanide, Toluene-2,4-diamine, Toluene-2,6-diamine, Toluene diisocyanate (TDI), Toxicology.

Sammanfattning

Montelius J (ed). *Vetenskapligt underlag för hygieniska gränsvärden.* XXII. Arbete och Hälsa 2001:20, s 1-96. Arbetslivsinstitutet, Solna.

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 2000 - juni 2001.

Nyckelord: Cyanväte, Difenylmetandiisocyanat (MDI), Etylentiourinämne, Hexametylendiisocyanat (HDI), Hygieniskt gränsvärde, Kaliumcyanid, α–Metylstyren, Natriumcyanid, Riskvärdering, Toluen-2,4-diamin, Toluen-2,6-diamin, Toxikologi, Toluendiisocyanat (TDI), Vetenskapligt underlag.

En svensk version av dessa vetenskapliga underlag finns publicerad i Arbete och Hälsa 2001:19.

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)
Cadmium	January 18, 1980	1981:21	(I)
revised	February 15, 1984	1984:44	(V)
revised	May 13, 1992	1992:47	(XIII)
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)
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)
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)
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)
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 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)
Methylene chloride	February 29,	1980	1981:21	(I)
4,4'-Methylene dianiline	June 16,	1987	1987:39	(VIII)
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)
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)
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)
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)
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	!989:32	(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)
Titanium dioxide	February 21,	1989	1989:32	(X)
Toluene	February 29,	1980	1981:21	(I)
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)
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)

Sent for publication December 2001