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CRITERIA DOCUMENTS FROM THE NORDIC EXPERT GROUP 1990

Brita Beije och Per Lundberg (Eds)

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PREFACE

The Nordic Council is an international body for the governments in the five countries, Denmark, Finland, Iceland, Norway and Sweden. One of the committees within the Nordic Council, the Nordic Senior Executive Committee for Occupational Environment Matters, initiated a project with a view to compiling and evaluating scientific information on chemical agents relevant to health and safety at work and the production of criteria documents. The documents are meant to be used by the regulatory authorities in the Nordic countries as a scientific basis for the setting of national occupational exposure limits.

The management of the project is given to a group of scientists: The Nordic Expert Group for Documentation of Occupational Exposure Limits. At present the Expert Group consists of the following members:

Helgi Gudbergsson	Municipal Institute of Public Health, Iceland
Per Lundberg (Chairman)	National Institute of Occupational Health, Sweden
Petter Kristensen	National Institute of Occupational Health, Norway
Vesa Riihimäki	Institute of Occupational Health, Finland
Adolf Schaich Fries	National Institute of Occupational Health, Denmark

The secretariat is located at the National Institute of Occupational Health, S-171 84 Solna, Sweden.

The criteria documents aim at establishing a dose-response/dose-effect relationship and a critical effect, based on published scientific literature. The task is not to give a proposal for a numerical exposure limit value.

The literature is evaluated and a draft is written by a scientist appointed by the Expert Group with the support and guidance of one member of the group. The draft is then sent for a peer review to experts by the secretariat. Ultimately the draft is discussed and revised at the Expert Group Meeting before it is accepted as their document.

Only studies considered to be valid and reliable as well as significant for the discussion have been referred to. Concentrations in air are given in mg/m^3 and in biological media in mol/l or mg/kg. In case they are given otherwise in the original articles they are, if possible, recalculated and the original values are given within brackets.

This volume consists of English translations of the criteria documents which have been published in a Scandinavian language during 1990. The names of the scientists who have written the separate documents are given in the list of contents, where aslo the dates of acceptance by the Expert Group are given.

Solna in December 1990

Brita Beije
Secretary

Per Lundberg
Chairman

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THIURAMS AND DIMETHYLDITHIOCARBAMATES

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Kuopio, November 15, 1989

Contents

Abbreviations:

Diethylamine = DEA
Diethyldithiocarbamate = DDC
Dimethyldithiocarbamate = DMDC
Dipentamethylenethiuram disulfide = PTD
Disulfiram = DSF
Ethylenebisdithiocarbamate = EBDC
Methyl diethyldithiocarbamate = MeDDC
Tetraethylthiuram disulfide = DSF
Tetramethylthiourea = TMTU
Tetramethylthiuram disulfide = TMTD
Tetramethylthiuram monosulfide = TMTM
2-Thiothiazolidine-4-carboxylic acid = TTCA
Thiram = TMTD

BACKGROUND

Thiurams and dimethyldithiocarbamates (DMDC), chemicals widely used as agricultural fungicides as well as accelerators and vulcanizers in the rubber industry, share common structural and toxicological properties. Disulfiram (DSF), ferbam, thiram (TMTD), and ziram will be discussed in this document.

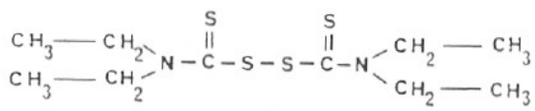
The use of thiurams and DMDC's has been declining because of their sensitizing properties. DMDC's have also been claimed to be mutagenic, teratogenic, and possibly carcinogenic (41, 166), and this has had a negative impact on the use of these compounds. Also, less harmful agents with a comparable fungicidal potency have been introduced into the market.

Considering the several toxicological drawbacks of thiurams and DMDC's their use is still widespread. No thorough evaluation of the toxicological significance of these compounds in the occupational environment has been recently carried out. Moreover, the present toxicological information renders re-evaluation of these compounds necessary (see 30, 157).

1 PHYSICAL AND CHEMICAL DATA

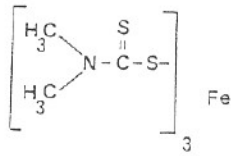
Disulfiram (DSF)

Chemical name	tetraethylthiuram disulfide
CAS number	97-77-8
Synonym	disulfiram, antabuse
Formula	$C_{10}H_{20}N_2S_4$
Structural formula	



Molecular weight	296.56
Melting point	70° C
Vapor pressure	nonsignificant
Density	1.30
General description	Yellowish odorless crystalline powder, practically insoluble in water (0.2 g/l), soluble in alcohol (38.2 g/l), in ether (71.4 g/l), also soluble in acetone, benzene, and carbon disulfide. Stable at room temperature.

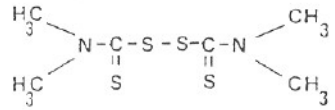
Ferbam (FeDMDC)

Chemical name	Iron dimethyldithiocarbamate
CAS number	14484-64-1
Synonym	ferbam, ferric dimethyldithiocarbamate
Formula	$C_9H_{18}N_3S_6.Fe$
Structural formula	

Molecular weight	416.51
Melting point	melts with decomposition above 180° C
Vapor pressure	nonsignificant
General description	Black odorless crystalline powder, practically insoluble in water (0.12 g/l), soluble in acetone, chloroform, pyridine, and acetonitrile. Stable at room temperature, decomposes > 180° C.

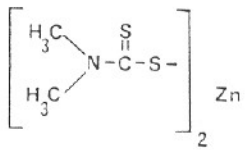
Thiram (TMTD)

Chemical name	tetramethylthiuram disulfide
CAS number	137-26-8
Synonym	thiram, bis(dimethylthiocarbamoyl) disulfide
Formula	$C_6H_{12}N_2S_4$

Structural formula 

Molecular weight	240.44
Melting point	155-156° C
Vapor pressure	nonsignificant
Density	1.29
General description	Colorless, odorless crystalline powder, insoluble (30 mg/l) in water, solubility in alcohol or ether less than 2 g/l, solubility in acetone 12 g/l, in benzene 25 g/l, more soluble in chloroform. Stable at room temperature, but decomposes readily under acidic or alkaline conditions and under prolonged exposure to air, heat or moisture. It is non-corrosive and non-explosive.

Ziram (ZnDMDC)

Chemical name	zinc dimethyldithiocarbamate
CAS number	137-30-4
Synonym	ziram, zinc bis(dimethylthiocarbamoyl) disulfide
Formula	$C_6H_{12}N_2S_4.Zn$
Structural formula	

Molecular weight	305.82
Melting point	148° C
Vapor pressure	nonsignificant
Density	(between 4 and 25° C) 1.66

General description Colorless, odorless crystalline powder, practically insoluble (65 mg/l) in water, solubility (at 25°C) <2 g/l in alcohol, carbon tetrachloride, or ether, < 5 g/l in acetone, benzene, or naphtha, more soluble in chloroform. Stable under normal conditions but decomposes in acidic media.

2 USES AND OCCURRENCE

2.1 Usage

DSF is used as an antialcoholic drug in addition to its use as an additive in the rubber industry, but in the Nordic countries it has no use as a disinfectant or as a fungicide. Ferbam is also used in the rubber industry but it is not used as a fungicide in any of the Nordic Countries. TMTD is used in all Nordic countries as a fungicide though its use is strictly limited. Ziram is used as a fungicide only in Denmark and Sweden. DSF is included in this document mainly because it is a useful model compound and because most of its potential toxic effects have been much better studied than those of any other thiurams or DMDC's.

2.2 Occupational exposure

There are no relevant data on the exposure of humans to DSF, ferbam, thiram or ziram.

2.3 Determination of thiurams and dimethyldithiocarbamates in air

Thiurams and DMDC's in the air can be collected by using PVC or cellulose ester membrane filters (114). The filter paper is placed with a filter back-up pad in a plastic cassette. The filter on the midget impinger is placed e.g. in workers breathing zone and connected with a tygon tubing to a personal sampling

pump. The compound is then collected on the filter paper. There are no state-of-the-art methods for the determination of all of these compounds in the air. For high pressure liquid chromatographic (HPLC) separation of TMTD and its two degradation products, notably TMTM and tetramethylthiourea (TMTU), a mixture of 45 % chloroform in cyclohexane and silica gel column (Separon SIX) can be used (6, 7). Moreover, TMTD in air can be detected by using total oxidation sulfur microcoulometry. The method involves a simple extraction, concentration, and a direct injection of the TMTD sample into the microcoulometer. A limitation is that no chromatographic separation is involved and that all sulfur-containing compounds in the sample being analyzed will give a response in the titration cell (8).

Palassis (115) has also described a method for the measurement of airborne TMTU (see above). The compound is collected by using a pump and midget impingers containing 15 ml of water. Pentacyanoamineferrate reagent is then added to the impinger contents to form a colored coordination complex. The absorbance of the solution is measured spectrophotometrically at 590 nm, and the unknown concentrations of TMTU in the samples are determined from calibration curves. The detection limit of this method is 3 µg/sample. Palassis (115) estimated that the method can be used as a general analytical method for the analysis of other thiourea-derived compounds. Most of the analytical methods available for thiurams and DMDC's have been developed for the determination of residues in crops (54) and concentrations in biological fluids (40). Applications of these methods can probably be used to measure thiurams, DMDC's and their degradation products in the air.

3 TOXICOKINETICS

3.1 Uptake

The lack of inhalational and dermal human or experimental animal exposure data renders the evaluation of these exposure routes for

the uptake of thiurams and DMDC's problematic. However, uptake via the lungs in the occupational environment is probably the most important. Dermal exposure and penetration through the skin, probably of minor significance, are also possible if a direct contact with skin and liquids containing thiurams or DMDC's takes place. Mainly experimental animal uptake data using oral or intraperitoneal (i.p.) routes are available.

3.1.1 Uptake by inhalation

No data of DSF, ferbam, TMTD or ziram are available concerning humans or experimental animals on uptake by inhalation.

3.1.2 Uptake through the skin

No data of DSF, ferbam, TMTD or ziram are available on humans or experimental animals.

3.1.3 Uptake from the gastrointestinal tract

Disulfiram. DSF is readily but incompletely absorbed from the human gastrointestinal tract. Hald and Jacobsen (55) found 20 % of orally administered DSF in the feces. Methyl diethyldithiocarbamate (MeDDC), an intermediate of DSF metabolism, appeared in blood one hour after oral administration of DSF to alcoholic patient volunteers (15, 16).

DSF and diethyldithiocarbamate (DDC) were demonstrated in blood, liver, kidney and muscle of rats two hours after oral dosing the absorbed amount being 70-90 % (25). Oral dose (2-50 mg/kg) of ^{14}C DSF to rats was mainly excreted in urine (87 %) and feces (7 %), and more than 80 % of the total dose within 48 hours. After 144 hours 95% of the ingested radioactivity was excreted in urine and feces with less than 1% in blood, organs, or carcass (107). Also, 144 hours after an oral dose of 50 mg/kg of ^{14}C and ^{35}S DSF to rats the radioactivity was found in urine (75 %), feces (13 %),

and expired air (6 %) (108). The absorption of DSF in rat seems to be more complete than in humans.

Ferbam. There are no data of the gastrointestinal absorption of ferbam in humans. About 40-70% of an oral dose of 500 mg/kg of ^{35}S ferbam was absorbed through the gastrointestinal tract of the rat during a 24 hour period. In these rats, 22.7, 18.1, and 1.0% of the radioactivity was found in urine, expired air, and bile, respectively. Only small amounts were found in tissues. In rats receiving ^{14}C ferbam, 42.9 and 1.4% of the radioactivity was found in urine and bile, respectively (62). Oral absorption of radioactive ferbam seems somewhat unpredictable in rats, and measured amounts depend on the quality and the place of the label in the molecule. Sheep received an oral dose of 0.45-0.74 mg/kg of ^{35}S or ^{3}H labeled ferbam. By 76 hours, 82% of the ^{3}H, and 23% of the ^{35}S moiety had been excreted in feces and urine. Significant amounts of radioactive ferbam appeared in urine or bile already 4 hours after oral administration in rats and sheep; ferbam seems to be readily but uncompletely absorbed (62, 65).

Thiram. There are no data of the gastrointestinal absorbtion of TMTD in humans. Zemaitis and Greene (170) found that 24 hours after oral administration of 1 g/kg of TMTD hepatic microsomal aniline hydroxylase and carboxylesterase activities were decreased indicating gastrointestinal absorption of TMTD in rats. Moreover, oral administration of 3.8 mg/kg of TMTD to rats significantly inhibited elimination of ethanol from the blood already at 90 min after TMTD administration (133) indicating that oral absorbtion of TMTD had taken place.

Ziram. There are no data of the gastrointestinal absorbtion of ziram in humans. When 4.9 mg/kg of ziram was given orally to rats 90 min before ethanol the concentrations of ethanol were elevated already at one hour after ethanol administration and this increase became statistically significant at 4 hours (133). Ziram may be readily though not completedly absorbed via the oral route.

In conclusion, available data indicates that discussed thiurams and DMDC's may be readily but not necessarily completely absorbed from the gastrointestinal tract.

3.2 Distribution

Disulfiram. No data are available of the distribution of DSF in humans (see 33). Because of its high lipid solubility DSF is mainly accumulated in lipids of tissues (33). After 144 hours of oral administration of ^{14}C DSF less than 1% of the dose was in the blood, liver, kidney, and carcass the remaining being in urine and feces (107, 108). The brain had the lowest concentrations of DSF and its metabolites. DSF and its metabolites have also been found in the thyroid, adrenals, pancreas, stomach, small and large intestine, muscle, testes, lung, spleen, and heart (37, 38, 39, 145, 146, 147).

Ferbam. No data of the distribution of ferbam in humans are available. After peroral administration of 500 mg/kg of radioactive (^{14}C and ^{35}S) ferbam to rats radioactivity was found at 24 hours in urine (42.9 % of the dose), feces (20%), gastrointestinal tract (9.8 %), bile (1.4 %), whole blood (0.9 %), liver (0.7 %), expired air (0.6%), kidneys (0.2%), muscle (2%), and brain (<0.13%). Moreover, radioactivity was also found in pregnant rats: placenta (1.6%), fetuses (1.2%), and amniotic fluid (0.9%). During lactation, radioactivity was found in mammary gland (1.8%) of rats, and in the stomach (1.1%) and urine (9.2%) of rat pups (62). By 76 hours after the administration of radioactive (^{3}H or ^{35}S) ferbam to sheep, traces of radioactivity were detected in adrenals, brain, fat tissue, heart, kidney, liver, muscle, spleen, and thyroid (65). In pregnant rats, a small but significant amount of ^{14}C ferbam readily reached the fetus via placenta. In lactating rats, after an oral dose of ^{14}C ferbam, radioactivity was transferred in milk to the pups and excreted in their urine (62). These data together indicate that ferbam does not have a tendency to accumulate in the tissues.

Thiram. There are no data of the distribution of TMTD in human tissues. Indirect evidence in rats indicates that TMTD is distributed to the liver where it has a strong inhibitory effects on the activity on the enzymes responsible for biotransformation of xenobiotics (20, 133, 170). Moreover, because the metabolism of TMTD is very similar to that of DSF (20) similarities in the distribution of TMTD and DSF are likely.

Ziram. There are no data of the distribution of ziram in human tissues. Ismirova and Marinov (69) found that 24 hours after oral administration of ^{35}S ziram to rats the radioactivity was mainly distributed to thyroid gland, blood, kidneys, spleen, ovaries, and liver. Also, in rats, strong effects of ziram in the liver 4 hours after its administration indicate that ziram is distributed in the liver (133).

In conclusion, none of the thiurams and DMDC's discussed seems to accumulate in the tissues. On the contrary, their excretion seems to be rapid as indicated by generally low tissue concentrations following administration.

3.3 Biotransformation

Disulfiram. The biotransformation of DSF proceeds first by a reduction of the disulfide linkage to its corresponding thiol-metabolite, DDC (33). This may lead to the formation of mixed disulfides with protein sulfhydryl groups. The glutathione reductase system of the erythrocytes effectively reduces DSF (75, 146, 147), and probably about 50 g of DSF can be reduced by adult human erythrocytes within 24 hours (145) causing a rapid disappearance of the parent compound from the blood stream (15, 16). DDC can be further metabolized via four different pathways, ie. glucuronidation, nonenzymatic degradation, methylation, and oxidation. Also, in urine of two persons given an oral dose of 250 mg of DSF small amounts of 2-thiothiazolidine-4-carboxylic

acid (TTCA) was identified. The amount of TTCA excreted in urine amounted 0.2-0.4 % of the dose (26).

Glucuronidation of DDC with glucuronic acid is the major detoxification pathway for DSF (72). About 50 % of radioactive DSF is excreted as a glucuronide conjugate in urine (146, 147).

Figure 1

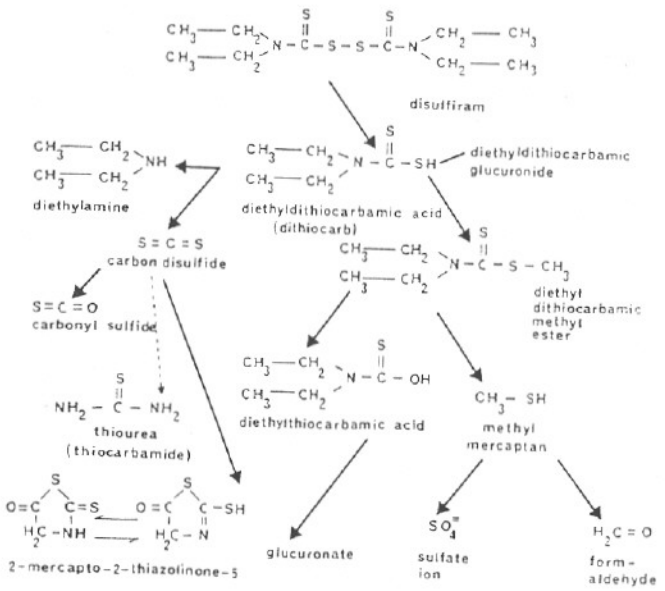


Figure 1. Metabolic pathways of disulfiram according to Gessner and Jakubowski (46) as well as Dalvi et al. (21) and Pergal et al. (118, 119).

The rate of the decomposition of DSF is also pH dependent (4), and in acidic medium DSF rapidly decomposes to diethylamine (DEA) and carbon disulfide (CS_2; 148). DEA may be excreted unchanged in man (107, 163) but it may also degrade to ammonia and acetaldehyde. In the presence of cytochrome P-450 CS_2 is oxidatively desulfurated to carbonyl sulfide (COS) and elemental sulfur (98).

One of the several metabolites of DSF, COS, is further oxidized to carbon dioxide (CO_2) and elementar sulfur (124, 125) (see Figure 1).

Methylation is a minor metabolic pathway for DSF in rodents because when ^{35}S DSF was administered i.p. to rats only 0.05 % of the dose was detected as a methyl ester of DDC (MeDDC) in urine (15, 16). However, in another experiment, where DDC was administered intravenously to dogs, S-methylation accounted for about 27 % of the metabolism of the dose (46).

Interestingly, small amounts of MeDDC have also been identified in urine of alcoholic patient volunteers between one and two hours after repeated 250 mg doses of DSF (15, 16). Methylated products can be attacked by esterases, generating methyl mercaptan which is further oxidized to sulfate and formaldehyde (15, 16). Moreover, small amounts of DDC can be reoxidized to DSF (146, 147). This reaction is, however, not of practical significance.

Ferbam. There are no data of the metabolism of ferbam in humans. Hodgson et al. (62) found that after absorbtion of 40-70 % of an oral dose of ^{35}S or ^{14}C ferbam in rat during a period of 24 hours a large part of ^{35}S ferbam was excreted in urine (22.7 % of the dose), expired air (18.1 %) or in bile (1.0 %). In rats receiving ^{14}C ferbam the radioactivity was excreted within 24 hours in urine (42.9 %), bile (1.4 %), and expired air (0.6 %). The only respiratory metabolite was CS_2. Major metabolites in urine included inorganic sulfate, a salt of a DMA and a glucuronide conjugate of DMDC. Unchanged ferbam was not found in the urine. In pregnant rats given ^{14}C ferbam a small but significant amount of the dose readily crossed the placenta into the fetus. Similar experiments with similar results have also been carried out using sheep (65).

Thiram. There are no data of the metabolism of TMTD in humans.

TMTD was excreted in a dose-dependent fashion as CS_2 in expired air after i.p. dosing to rats. However, only 0.5 % of the dose of TMTD was exhaled as CS_2 (20). In vivo and in vitro experiments with rats also provided evidence that TMTD is metabolized to DMDC and CS_2 (170).

Ziram. There are no data of the metabolism of ziram in humans. However, in one study where ziram was given perorally to rats and mice (500 mg/kg) it was metabolized to TMTD, TMTU, DMA salt of DMDC, CS_2, and DMA (159).

3.4 Elimination

3.4.1 Elimination by inhalation

Disulfiram. Faiman et al. (40) reported that 22.4 and 31.3 % of the oral doses DSF was eliminated via exhaled air as CS_2 after one or several doses when the single dose was 250 mg, respectively. The apparent half life for the elimination of CS_2 in exhaled air was 8.9 hours. When 50 mg/kg of ^{35}S DSF was given to rats by gavage 6 % of the administered dose was eliminated via exhaled air, probably as CS_2 (108). However, when ^{35}S MeDDC was administered i.p. to male rats, no radioactivity was detected in exhaled air (38). In another study with rats, about 12 % of orally administered dose of 7 mg/kg of ^{35}S DSF was exhaled as CS_2 (37).

Ferbam. There are no data of the elimination of ferbam via lungs in humans. In rats receiving ^{35}S ferbam 18.1 % of the radioactivity was found in expired air (62).

Thiram. There are no data of the elimination of TMTD via lungs in humans. However, Dalvi and Deoras (20) reported that about 0.5 % of orally administered dose of 200 mg/kg of TMTD was excreted in expired air as CS_2.

Ziram. There are no human or animal data of the excretion of ziram via lungs.

3.4.2 Elimination by the kidneys

Disulfiram. Faiman et al. (40) reported that after a single or repeated oral doses of 250 mg of DSF to humans, 1.7 and 8.3 % of the dose was eliminated as DDC-glucuronide in the urine while DEA accounted for 1.6 and 5.7 % of the dose, respectively.

When dogs were given DDC, 27 % of the dose was S-methylated. MeDDC was then eliminated with a half life of 49.2 min from the plasma (17). In rats, 66.8 % of oral dose of ^{35}S DSF was excreted in urine within 48 hours, mainly as DDC glucuronide or inorganic sulfate (37). Also, in mice and dogs, ^{35}S DSF was rapidly eliminated in urine mainly as DDC-glucuronide and inorganic sulfate, even though also DSF and MeDDC were found in urine after i.p. administration of ^{35}S DSF in these species (39). Following the administration of ^{35}S MeDDC to male rats most of the total radioactivity was found in urine within 12 hours. When ^{14}C DSF was given to male rats by gavage 87 % of the radioactivity was found in urine. The major urinary metabolites of DSF were DEA and DDC-glucuronide (107). Similar results were obtained in another study with rats (108).

Ferbam. There are no data of the urinary excretion of ferbam in humans. However, in rats receiving ^{35}S ferbam 22.7 % of the radio-activity was excreted in urine (62). In sheep, 82 % and 23 % of ^{3}H and ^{35}S moieties were excreted in urine and feces by 76 hours (65).

Thiram and Ziram. There are no human or experimental animal data of the urinary excretion of TMTD or ziram.

3.4.3 Elimination through the gastrointestinal tract

Disulfiram. There are no data of the excretion of DSF in feces in humans. Of perorally administered ^{35}S DSF to rats about 6,5 % was excreted as DSF in feces within 48 hours after the administration (37). However, 53.2 % the radioactivity of DDC-glucuronide, the product of the main metabolic pathway of DSF, was found in gastrointestinal tract. According to Faiman et al. (37) this emphasizes the significance of enterohepatic circulation in the excretion of DSF. In another study with rats, 13 % of the DSF radioactivity was excreted in feces within 144 hours after the administration of ^{35}S DSF (108). Similar findings were reported by Neiderhiser and Fuller (107), also in rats. When ^{35}S MeDDC was administered i.p. to rats the feces accounted for 15 % of the excreted radioactivity (38). The data indicates that a significant portion of absorbed DSF is excreted as DDC-glucuronide in those cases where it has been studied emphasizing the significance of enterohepatic cycling in the excretion of DSF.

Ferbam. There are no data of the gastrointestinal elimination of ferbam in humans. However, in rats receiving ^{35}S ferbam 1.0 % of the radioactivity was found in bile. Likewise, in rats receiving ^{14}C ferbam the amount of the dose of ferbam excreted into the bile accounted only 1.4 % of the whole dose (62).

Thiram and Ziram. There are no data of the gastrointestinal elimination of TMTD or ziram in humans or experimental animals.

3.4.4 Biological half lives

Disulfiram. Elimination kinetics of DSF were determined in 15 male alcoholics after 250 mg of DSF taken by mouth as a single dose and again after 12 days of repeated dosing. Apparent half lives in plasma were calculated for DSF, DDC, MeDDC, DEA, and CS_2, and they were 7.3, 15.5, 22.1, 13.9, and 8.9 hours, respectively. Elimination half life for CS_2 in breath was 13.3 hours. Average time to reach maximal plasma concentration after either single or repeated doses was 8-10 hours for DSF, DDC, MeDDC, DEA, and CS_2 in breath, while plasma CS_2 peaked at 5-6 hours after the administration of 250 mg of DSF (40).

No such data in humans or experimental animals were available for ferbam, TMTD, or ziram.

3.5 Biological monitoring

Disulfiram. There are not widely accepted biological indicators of exposure to DSF. However, several exposure indicators can be used in principle for the biological monitoring of exposure to DSF. Because DSF readily reacts with copper ion, colorimetric methods can be used for the determination of DSF in biological fluids (39). DSF can be determined in blood and in urine by gas chromatography (GC) or HPLC (40, 96, 135). Moreover, several metabolites of DSF, notably DDC, MeDDC, DEA, and CS_2 can be measured in plasma, urine, or exhaled air by using GC or HPLC methods (40, 88, 94, 96, 135). Also, glucuronide conjugates of DDC have been identified by using a GC/mass spectrometer combination (32).

The most promising of these methods for biological monitoring, namely applications of GC or HPLC need, however, further development. The elimination half lives of most of the metabolites of DSF and other thiurams and DMDC's in humans, 7-22 hours, are also well applicable for biological monitoring.

Both DSF and DDC inhibit erythrocyte aldehyde dehydrogenase (58, 67). This inhibition could possibly be used for biological monitoring of exposure to DSF, other thiurams, and DMDC's as well.

Ferbam. There are not readily available methods for the monito-

ring of ferbam in humans in occupational environment. Ferbam is, however, metabolized to TMTD in the body, and thus monitoring of TMTD metabolites can be applied for the monitoring of exposure to ferbam (see below). Moreover, ferbam can also be readily detected in agricultural products by using methods based on HPLC techniques (6, 7).

Thiram. There are not readily available methods for the monitoring of TMTD in biological fluids. TMTD and its metabolites DMDC and CS_2 can be, however, readily detected in food by using colorimetric methods (117, 160). TMTD and its metabolites in food stuffs can also be measured by GC and HPLC methods (7, 53, 54). Applications of these methods may provide the basis for biological monitoring methods of individuals exposed to TMTD.

Ziram. There are no readily available methods for the monitoring of ziram in biological fluids. However, ziram is metabolized to TMTD in the body (41) and, thus, methods which can be used for biological monitoring of TMTD also apply to the monitoring of exposure to ziram. For monitoring of thiurams and DMDC's, see also Slade (144).

There is not enough knowledge for biological monitoring of these agents which would be based on their pharmacokinetics. Therefore, not only analytical techniques for these agents but also their pharmacokinetics should be studied in occupational environments for the development of appropriate sampling strategies for biological monitoring.

4 GENERAL TOXICOLOGY

4.1 Toxicological mechanisms

The toxicological mechanisms of the various toxic effects of thiurams and DMDC's are not well known.

The reduction of the disulfide bond of DSF and other thiurams by glutathione SH-containing compounds leads to the formation of mixed disulfides which in turn may inhibit several enzymes (33). DSF, other thiurams and their metabolites such as DDC are potent chelators of copper and other metals, and this chelation may lead to the inactivation of enzymes. Zinc-containing aldehyde hydrogenase and glyceraldehyde phosphate dehydrogenase as well as copper-dependent enzymes, aldehyde oxidase and dopamine-ß-hydroxylase (see 33) belong to these enzymes. This capacity of thiurams to inhibit various enzymes may explain their capacity to inhibit their own metabolism as well as the metabolism of several other compounds such as ethanol (see 33).

Inhibition of dopamine-ß-hydroxylase has been claimed to be the reason for various central nervous system (CNS) effects of thiurams and DMDC's, ie. marked increases of serum TSH levels (11, 82). This enzyme catalyzes the conversion of dopamine to norepinephrine, and thus has a major effect on the metabolism of important neurotransmitters within the brain (11, 33).

DSF and other thiurams have also effects on other organ systems such as the liver and the brains as well as peripheral nerves. DSF and DMDC's are metabolized to CS_2, COS and finally also to hydrogen sulfide (H_2S), a potent inhibitor of oxidative phosphorylation (12, 13). Thus, one of the plausible mechanisms of thiuram intoxication in the brain (137) and other tissues (13) is decreased protein phosphorylation subsequent to decreased oxidative metabolism.

However, these observation do not explain the mechanism of polyneuropathy induced by DSF and other thiurams. Distal axonopathy produced by e.g. DSF and CS_2 has been claimed to be due to binding of metabolites of thiurams to neurofilaments of peripheral nerves or spinal cord (136, 140). Neuronal swelling and some myelin figures were seen in sciatic nerves of rats exposed to DSF, ferbam, TMTD, and ziram (86, 139). Also, when ferbam, TMTD

and ziram were added to mouse C1300 neuroblastoma cells they caused significant perinuclear accumulation of cytoskeletal structures within the cells. These results together suggest that the ultimate mechanism of peripheral neuropathy induced by thiurams and DMDC's may be due to an interaction between their metabolites and cytoskeletal structures of the nerves (86).

4.2. Factors affecting the metabolic model

Single doses of DSF and DDC inhibit rat small intestinal and liver benzo(a)pyrene mono-oxygenase activity (51) but 5-30 subsequent daily doses of DSF or DDC increase both benzo(a)pyrene hydroxylase and cytochrome P-450 oxidase activity (51, 52). TMTD and DMDC, DSF, DDC, and ziram inhibit several microsomal enzyme activities (43).

It is, therefore, not surprising that thiurams and their metabolites also have significant effects on the biotransformation of several other compounds. DSF (33), TMTD, tetramethylthiuram monosulfide (TMTM), and ziram all inhibit the biotransformation of ethanol (133).

Several of thiurams and DMDC's markedly increase the uptake of cadmium (9, 23, 44, 45), lead (110), nickel (70), and mercury (23). They also affect the distribution and elimination of heavy metals (1, 23, 24, 44, 45, 70, 142).

4.3 Acute toxicity

Systematic studies have been carried out on the acute toxicity of thiurams and DMDC's. In table 1 peroral LD_{50} values in rodents have been given for DSF, ferbam, TMTD and ziram. Also, the corresponding reference has been indicated.

Table 1. Peroral LD_{50} values for DSF, ferbam, thiram and ziram in rats, mouse and rabbit.

Compound	Species	LD_{50} (mg/kg)	Reference
Disulfiram	Rat	8600	101
Ferbam	Rat	2700-3500	60, 85, 165
	Mouse	3100-3700	85
Thiram	Rat	375-865	42, 167
	Mouse	2300-4500	41, 42, 85
	Rabbit	210	42
Ziram	Rat	500	165

5 ORGAN EFFECTS

5.1 Effects on skin, mucous membranes and eyes

Disulfiram, ferbam, thiram and Ziram. Besides allergic effects there are no human or animal data on the effects of DSF, ferbam, thiram or ziram on skin or mucous membranes (see section 6).

5.2 Effects on lungs

There are no human or animal data on the toxicity of disulfiram, ferbam, thiram in lungs.

Ziram. Workers exposed to 1.6-2.2 mg of ziram/m^3 had increased incidence of diseases of the upper respiratory tract such as rhinitis, laryngitis, and pharyngitis, bronchitis, and emphysema of the lungs (34, 35, 36, 95). The reliability of these studies was, however, difficult to judge because of imcomplete reporting.

Guinea pigs died after 1-6 subsequent 4-hour ziram exposures when the concentration of ziram exceeded 20 mg/m^3. A single 4-hour inhalation exposure to ziram at concentrations exceeding 250 mg/m^3

resulted in hemorrhage in the lungs and death. In mice and rats the LC_{50} values for ziram after a 4-hour inhalation exposure were 25 and 50 mg/m^3, respectively (99). The LC_{50} value of ziram for rabbits was 14.7 mg/m^3 when the animals were exposed 4 hours daily for 30 days (35). Long-term inhalation exposure of rabbits to ziram was associated with inflammation of the respiratory tract, interstitial pneumonia, and degeneration of the ciliated epithelium in the trachea (35).

5.3 Effects on gastrointestinal tract

Disulfiram. There are no data of the effects of DSF in the gastrointestinal tract of humans.

Repeated daily doses (220-245 mg/kg) of DSF for 21 days caused inflammation of intestinal mucosa and decreased the responsiviness of isolated ilea of the exposed rats to 5-hydroxytryptamine indicating a DSF-induced damage in the nerve plexuses of the intestinal wall. In agreement with this, DSF also decreased the histochemical reactivity of cholinesterases in the intestinal wall of the exposed rats (138, 139).

There are no data of the effects of ferbam, thiram or ziram in the gastrointestinal tract of humans or experimental animals.

5.4 Effects on liver

Disulfiram. DSF has been shown to cause hepatotoxicity in patients (31, 74, 126). Goyer and Major (50) did not find dose-dependent hepatotoxicity in DSF-treated patients, but some of the patients in their study had non-dose-related hepatotoxicity. These and other (106) investigators have claimed DSF-induced hepatotoxicity to be an idiosyncratic or an immunological reaction. Moreover, DSF has induced hypercholesterolemia in rats, at least in part due to a marked increase in the activity of hepatic hydroxy-ß-methyl glutaryl coenzyme A reductase (HMG-CoA

reductase), a rate-limiting step in cholesterol biosynthesis (131). DDC has a similar effect in rabbits (19).

Ferbam. There are no data of the effects of ferbam on the liver in humans. In several long-term animal studies, ferbam has not caused liver damage or alterations in liver functions (61, 85).

Thiram. There are no data of the effects of TMTD on the liver in humans. TMTD and its metabolite DMDC impair hepatic biotransformation by inhibiting liver microsomal aniline hydroxylase and carboxylesterase activities (170). TMTD also causes a significant loss in liver cytochrome P-450 and benzphetamine N-demethylase activities, presumably due to CS_2 formation which may be partly responsible for TMTD-induced hepatotoxicity (20). A single dose of 120 mg of TMTD/kg markedly increased serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities in rats indicating a liver damage (22). Piechocka (120) made similar findings, also in rats. Additional evidence for TMTD-induced liver damage was provided by the finding that there was a significant decrease in the activities of several drug metabolizing enzymes in the liver of rats (22). Lowy et al. (91) found that a daily dose of 20 mg/kg of TMTD induced liver enlargement and liver protein labeling in rats during 29 days' exposure. TMTD also caused liver enlargement in female rats at daily doses of 67 mg/kg after 80 weeks' exposure (85). However, Hodge et al. (61) did not observe TMTD-induced liver damage in rats in a two-year feeding study. Because ferbam and ziram are metabolically biotransformed to TMTD these observations may also apply to them.

Ziram. There are no data of the effects of ziram on the liver in humans. Hodge et al. (61) could not find ziram-induced liver damage in a two-year feeding study with rats.

5.5 Effects on kidneys

Disulfiram. There are no data of the effects of DSF on kidneys in humans. DSF has not caused deleterious effects in kidneys of experimental animals either (33).

Ferbam. There are no data of the effects of ferbam on kidneys in humans. Hodge et al. (61) did not find kidney damage in rats fed for two years with a diet containing up to 2500 mg of ferbam/ kg diet (125 mg/kg/day).

Thiram. There are no data of the effects of TMTD on kidneys in humans. TMTD has not caused damage in kidneys in rats exposed to TMTD for 29 days - 2 years (61, 85, 91). Korablev and Evets (80) reported that TMTD and TMTD derivatives had a marked antidiuretic action due to the inhibition of urinary excretion of sodium, potassium and water in rats and dogs.

Ziram. There are no data of the effects of ziram on kidneys in humans. Ziram did not damage kidney during a two-year feeding study at a dose of 125 mg of ziram/kg/day (61).

5.6 Effects on blood and blood forming organs

Disulfiram. DSF effectively inhibits erythrocyte aldehyde dehydrogenase activity (58, 67, 154). There are no reports of effects of DSF on blood or blood forming organs in humans or experimental animals.

Ferbam. There are no data of the effects of ferbam in blood or blood forming organs in humans. No deleterious effects by ferbam on blood or blood forming organs have been found in experimental animals (85).

Thiram. There are no data of the effects of TMTD in blood or blood forming organs in humans. No deleterious effects by TMTD on

blood or blood forming organs in experimental animals have been reported (61, 85).

Ziram. There are no well documented data of the effects of ziram in blood or blood forming organs in humans. No deleterious effects have been found in experimental animals (61).

5.7 Effects on central nervous system

Disulfiram. Extensive exposure, as in clinical medicine, to DSF may provoke psychopathological reactions such as psychosis (33, 87). Such exposure is not a plausible occurrence in occupational environment. Rainey (124) has compared the effects of DSF and CS_2 on the central nervous system (CNS) and has suggested that the effects of DSF on the CNS are mediated via CS_2. Heavy exposure to CS_2 has been associated with neurotoxic disorders (124, 125).

In rodents, DSF and DDC have also depressant effects characterized by ataxia, sedation, and hypotonia, depression of locomotor activity, and disruption of active and passive avoidance as well as operant behavior (10, 48, 102, 149). Both DSF and DDC also reduced orientation hypermotility and depressed subcortical EEG activity in rats (153). Ueno et al. (155) found pathological changes in hypothalamic neurons after a short-term exposure to DSF and damage of cortical neurons after 6 weeks' exposure to DSF at a daily i.p. dose of 100 mg/kg. Degenerative changes were found in cerebellum, medulla and spinal cord of rabbits after exposure to six weekly oral or i.p. doses of 330 mg of DDC/kg x day for 6-24 weeks (29, 129).

Puglia and Loeb (123) observed that DDC inhibited brain superoxide dismutase activity. These data indicate that DSF and its metabolites may disturb brain functions and cause brain damage by disturbing brain oxidative metabolism, and by increasing the production of active oxygen species, in addition to the inhibition of dopamine-ß-hydroxylase and subsequent alterations in brain

monoamine metabolism (see 33). However, DSF did not change significantly dopamine-(D_2)-receptor binding in brain of rats at doses which were overtly toxic (79).

At cellular level, DSF caused deleterious effects in the cytoskeleton of cultured murine C1300 neuroblastoma cells (86). All serious in vitro nerve cell (neuroblastoma cells) effects of DSF or its metabolites have been observed, however, at high exposure concentrations (1-10 µg/ml) of active compound.

DSF also causes a 3-6 fold increase in the accumulation of lead into the CNS, presumably due to the formation of lipid-soluble complex with the metal. This effect of DSF greatly enhances lead-induced neuronal depression and may be a cause for potentially dangerous interactions between lead and DSF, other thiurams, and DMDC's (110, 111, 112, 113).

Ferbam. There are no data of the effects of ferbam on the human CNS. In rats, 96 mg of ferbam/kg caused hyperactivity and ataxia, followed by a loss of muscular tone, decreased motor activity, and clonic convulsions (62, 84, 85). Hodge et al. (61) also found that ferbam caused cystic brain lesions during a two-year feeding study at a dose of 125 mg of ferbam/kg/day. These rats also had clonic convulsions. In the same study, 25 mg of ferbam/kg daily for one year caused clonic convulsions in young beagle dogs. Four daily doses of 60 mg/kg of ferbam did not change brain D_2-receptor binding (79), but it caused perinuclear accumulation of microtubules and intermediate filaments in cultured murine C1300 neuroblastoma cells (86).

Thiram. There are no data of the effects of TMTD on the human CNS. In rats, TMTD, at a daily dose of 67 mg/kg, altered behavior, caused ataxia and paralysis of the hind legs. Moreover, degeneration of spinal cord also occurred (84). A single dose of 240 mg of TMTD/kg orally reduced orientation hypermotility and depressed subcortical EEG (153) but four subsequent daily i.p.

doses of 20 mg of TMTD/kg did not alter brain D_2-receptor binding in rats (79). It caused, however, dense perinuclear accumulations of microtubules and intermediate filaments in cultured murine C1300 neuroblastoma cells (86).

Ziram. There are no data of the effects of ziram on the human CNS. In a two-year feeding study with rats, ziram caused behavioral alterations at a dose of 125 mg/kg/day. In the same study, ziram caused clonic convulsions in young beagle dogs at a dose of 25 mg/kg/day (61). Moreover, ziram has caused perinuclear accumulation of microtubules and intermediate filaments in cultured murine neuroblastoma C1300 cells (86).

Because much of the data obtained from studies with DSF probably apply also to other thiurams and DMDC's all of them may have effects on brain oxidative and monoamine metabolism. Based on in vitro evidence, they may also have the capacity to cause changes in the cytoskeleton of neural cells. These findings may provide the basis for the mechanisms of the known neurotoxic effects of thiurams and DMDC's.

5.8 Effects on peripheral nervous system

Disulfiram. There are several cases in which DSF therapy has caused distal polyneuropathies in patients (5, 33, 93, 102, 103, 104, 109, 161). A typical feature of DSF polyneuropathy is the loss of myelinated fibres in the peripheral nerve. Several of the remaining myelinated fibres are undergoing Wallerian type axonal degeneration; large myelinated fibres are mainly involved. There seems also to be a difference between DSF- and CS_2-induced dying-back polyneuropathy and, therefore, some investigators do not consider CS_2 responsible for DSF polyneuropathy (see 5, 140).

Anzil and coworkers (3, 171) noted that DSF causes a dying-back type polyneuropathy in rats. Savolainen et al. (139) and Lehto et al. (86) observed that DSF decreases the number of microtubuli in

the sciatic nerve of rats. These findings are consistent with the finding that DSF may damage cytoskeletal structures in cultured murine C1300 neuroblastoma cells (86). DSF also decreases the responses of isolated ileal preparations of rats exposed to DSF for several weeks to 5-hydroxytryptamine, and decreases the histochemical reactivity of the intestinal wall cholinesterases indicating a DSF-induced damage to the autonomic innervation of the intestine (138, 139).

Ferbam. There are no data of the effects of ferbam in the peripheral nerves in humans. In rats, long-term oral administration of ferbam causes ataxia and paralysis on hind legs (61, 84). Oral exposure to ferbam was associated with axonal swelling of sciatic nerve fibers in rat. Also, ferbam caused perinuclear accumulation of microtubules and intermediate filaments in murine C1300 neuroblastoma cells (86).

Thiram. There are no data on the effects of TMTD in peripheral nerves in humans. In rats, TMTD caused ataxia and paralysis of hind legs in rats in experiments which lasted 36-104 weeks (61, 84). Demyelination was observed in the fibers of the sciatic nerve (84). A short-term exposure to 10-30 mg/kg/day for 4 days caused slight axonal swelling in the sciatic nerve of rat. TMTD also caused perinuclear accumulation of microtubules and intermediate filaments in murine C1300 neuroblastoma cells (86).

Ziram. There are no data of the effects of ziram in peripheral nerves in humans. Exposure to ziram for two to six months caused ataxia and paralysis of hind legs in rats (61). Two subsequent daily doses of 10 mg of ziram/kg produced myelin figures in sciatic nerves as an early indication of degenerative changes in the nerve. Parallel experiments showed that ziram induced perinuclear accumulation of cytoskeletal structures in cultured murine C1300 neuroblastoma cells (86).

5.9 Effects on thyroid gland and hypophyseal functions

Disulfiram. DSF, when given as a 1000 mg single dose to male and female volunteers, decreased serum thyroid stimulating hormone (TSH) and prolactin levels in males and females whereas serum luteinizing hormone levels and follicle stimulating hormone levels were decreased only in females or males, respectively. It was suggested that inhibition of brain dopamine-ß-hydroxylase may be behind this action of DSF (11). The effects of DSF on TSH are in agreement with the findings in maneb- and zineb-exposed rats after cold stimulation (83) but contradictory to the findings in rats exposed to another EBDC, nabam, in basal conditions (82). The mechanism of the action of DSF on TSH and other pituitary hormones thus remains unexplained. Telkkä and Kivalo (150) found, however, that 30 daily doses of 25 mg of DSF/kg induced marked histological hyperplasia and weight increase in thyroid gland in rats, plausibly due to the inhibition of the synthesis of thyroid hormones.

Ferbam. There are no data of the effects of ferbam on thyroid gland or hypophyseal functions in humans. Ferbam has not been reported to cause thyroid hyperplasia in rodents (61, 85) but it increased the incidence of thyroid metaplasia after two years of exposure in rats (85).

Thiram. There are no data of the effects of TMTD on thyroid gland or hypophyseal functions in humans. Lowy et al. (91) did not find thyroid hyperplasia in rats after 29 days' exposure at daily doses between 20 and 50 mg of TMTD/kg but Lee et al. (85) observed that a daily dose of 52 and 67 mg of TMTD/kg for 80 weeks to male and female rats, respectively, clearly increased the incidence of thyroid metaplasia and increased relative thyroid weights in exposed rats.

Ziram. There are no data of the effects of ziram on thyroid gland

or hypophyseal functions in humans. Ziram did not induce clear-cut alterations in thyroids of rats exposed to a daily dose of 125 mg/kg for two years (61).

6 IMMUNOTOXICITY AND ALLERGY

Generally, thiurams and DMDC's are known as strong allergens (164). There are no data on their immunotoxic properties.

Disulfiram. DSF has not been proved sensitizing as such but it may cause a severe flare-up of allergic contact dermatitis in nickel- (71) and cobalt-sensitive patients (100).

Ferbam. There are no data of the sensitizing properties of ferbam in humans. Ferbam is, however, a strong sensitizer in guinea pigs, and it cross-reacts with other thiurams and DMDC's such as TMTD and ziram (97).

Thiram. Workers and surgical staff occupationally exposed to TMTD via gloves have experienced allergic contact dermatitis after repeated exposures (77, 78, 90). Also, an oral exposure may lead to dermal sensitization to TMTD (49) and delayed cutaneous allergic reactions have also been reported (76). The incidence of TMTD hypersensitivity has been reported to be higher among men than women (151). TMTD is a strong dermal allergen in guinea pig, and it cross-reacts with other thiurams and DMDC's (97). There is also one case-report on a uncommon but serious systemic hypersensitivity reaction (Henoch-Schönlein purpura), possibly caused by TMTD, consisting of abdominal pains, generalized arthralgias, and progressive purpuric rash. The condition improved after the cessation of the exposure and initiation of corticosteroid therapy (27).

Ziram. Ziram is a strong contact allergen in humans which cross-reacts with a variety of other thiurams and DMDC's (49, 77, 132). Ziram also causes strong allergic dermatitis in guinea pigs, and

it cross-reacts with other thiurams and DMDC's (97).

7 GENOTOXIC EFFECTS

Disulfiram. There are no data of the genotoxic effects of DSF in humans. DSF proved not to be clastogenic in rats (18). Hedenstedt et al. (57) and Hemminki et al. (59) found that DSF was non-mutagenic in Salmonella typhimurium mutagenicity tests with and without metabolic activation when strains TA1535 and TA100 for base-pair substitutions were used. On the contrary, Rannug et al. (128) found that DSF was mutagenic when studied with Salmonella typhimurium tester strains TA1535, TA1537, TA1538, TA98 and TA100 in the presence of metabolic activation. Thus, the question of the genotoxicity of DSF remains somewhat controversial eventhough there is some evidence for the genotoxicity of DSF.

Ferbam. There are no data of the genotoxic effects of ferbam in humans. Ferbam was mutagenic with and without metabolic activation with Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537, and TA1538 and a strain (WP2 hcr) of Escherichia coli. Ferbam was also very toxic in cultures of chinese hamster ovary cells (CHO-K1 cells) having LC_{50} values of 7×10^{-7} M (63). Thus, there is evidence that ferbam is cytotoxic in in vitro systems and may have genotoxic potential in bacterial test systems.

Thiram. There are no data of the genotoxic effects of TMTD in humans. TMTD has proved, however, very mutagenic in test systems using Salmonella typhimurium tester strains with and without metabolic activation (2, 57, 59, 73, 105, 127, 128, 168, 169). There is convincing evidence that TMTD is both direct- and indirect-acting mutagen and that it induces both frameshift and base-pair mutations in bacterial test systems (127).

TMTD induced a significant increase in the frequency of chromosomal aberrations and the number of abnormal sperms in mice at all

tested doses (80, 200, or 320 mg/kg) of TMTD (122). An i.p. dose of TMTD of 500 or 1000 mg/kg increased the incidence of micronuclei in polychromatic erythrocytes in bone marrow of mice while 100 mg/kg was ineffective (28, 116) and also sperm abnormalities were observed after i.p. administration of TMTD (30-100 mg/kg) to mice (168). TMTD induced morphological alterations in the chromosomes of Aspergillus nidulans as well (156). In cytotoxicity tests with CHO-K1 cells the LC_{50} value for TMTD was 5×10^{-7} M (63).

Ziram. The number of chromosomal aberrations was increased in the peripheral leukocytes of workers exposed to ziram. The frequency of aberrated leukocytes was 0.75 % in control and 5.9 % in the exposed group, respectively (121). Also, ziram caused mutagenic effects in Salmonella typhimurium tester strains with and without metabolic activation (57, 59, 105, 128). Thus, there seems to be little doubt that ziram has a remarkable genotoxic potential, at least in bacterial test systems.

8 CARCINOGENICITY

Disulfiram. There is no information regarding the carcinogenicity of DSF in humans. DSF has not been carcinogenic in rats or mice (66, 68, 157).

Ferbam. There is no information of the carcinogenicity of ferbam in humans (68, 157). Ferbam has not been carcinogenic in rats or mice (61, 66, 68, 85, 157).

Thiram. There is no information of the carcinogenicity of TMTD in humans (68, 157). TMTD has not been carcinogenic in rats or mice (56, 66, 68, 85, 89, 157).

Ziram. There is no information of the carcinogenicity of ziram in humans (68, 157). Ziram has not shown any carcinogenic potential in several studies with mice or rats (61, 66, 68, 157). However, it was found in the National Toxicology Program (64) that ziram

dose-dependently increased the incidence of C-cell carcinomas of the thyroid in male but not in female F344 rats. Ziram increased, also dose-dependently, the number of alveolar or bronchiolar adenomas in female, but not in male, $B6C3F_1$ mice. The latter finding was, however, complicated by a concomitant Sendai virus infection in all groups, controls included.

Generally, the information of the carcinogenicity of thiurams and DMDC's inadequate according to IARC criteria (68). Thus, at the present state of knowledge, it is not possible to evaluate the carcinogenic potential of these compounds.

9 REPRODUCTION AND TERATOGENICITY

9.1 Effects on reproduction

Disulfiram. There are no data of the effects of DSF on gonads or fertility in humans (33). DSF reduced the fertility of laying hens at all tested doses (125-500 mg of DSF in kg of diet) given for 7 days (162).

Ferbam. There are no data of the effects of ferbam on gonads or fertility in humans. Ferbam administered in the diet at a daily dose level of 51 mg/kg did not affect the fertility of male or female mice (143). Ferbam caused a dose-dependent decrease in egg production and the weight of ovaries when given to laying hens after a single or 7 repeated doses at a dose level of 2.5 mg/kg x day or more while 0.5 mg/kg x day was ineffective (141, 162). The antifertility effect correlated with the inhibition of dopamine-ß-hydroxylase (141).

Thiram. There are no data of the effects of TMTD on gonads or fertility in humans. Inhalation exposure of female rats to 1 mg/m^3 of TMTD for 4 months decreased the number of follicles, and ovocytes. A similar exposure of male rats caused depressed spermatogenesis and decreased sperm motility (158). Oral exposure of

male rats to TMTD for 13 weeks at a daily dose of 132 mg/kg caused moderate testicular tubular degeneration and atypical spermatids occurred (85). A 30-day oral exposure of male rats to TMTD caused a dose-related decrease in the weights of testes (91, 92). TMTD, given in the diet for 13 weeks at a daily dose of 132 mg/kg, produced infertility in male mice and delayed the female estrous cycle at a daily dose of 96 mg/kg given for a similar period (143). TMTD also decreased fertility and the weight of ovaries in laying hens between daily doses of 2.5-20 mg of TMTD/kg. These effects in hens correlated with the inhibition of dopamine-ß-hydroxylase (141, 162).

Ziram. There are no data of the effects of ziram on gonads or fertility in humans. In laying hens, ziram decreased egg production and the weights of the ovaries in a manner which correlated with the inhibition of dopamine-ß-hydroxylase at doses 0.5-20 mg/kg x day (141, 162).

9.2 Embryotoxic and teratogenic effects

Disulfiram. There are no data of the embryotoxicity or teratogenicity of DSF in humans. DSF induced in pregnant rats at a daily dose of 100 mg/kg during pregnancy an increased amount of early resorptions (134). A similar finding was made in mice at a daily dose of 10 mg/mouse (about 400-500 mg/kg) (152). DSF also increased fetal mortality and the number of terata when given in DMSO to hamsters during the pregnancy. This effect was not observed when DSF was given in carboxymethyl cellulose (130). DSF increased the number of fetal deaths when tested in eggs (81). In chicken, DSF also induced tibial dyschondroplasia (30). There is, thus, some evidence of avian and rodent embryotoxic effects of DSF.

Ferbam. There are no data of the embryotoxic or teratogenic effects of ferbam in humans. In mice, a daily dose of 114 mg/kg of ferbam during pregnancy reduced the maternal weight gain, litter size, and fetal body weight (143). Fetotoxicity was only seen at doses also toxic to mothers.

Thiram. There are no data of the embryotoxic or teratogenic effects of TMTD in humans. In mice, TMTD treatment during pregnancy reduced maternal weight gain and fetal weight when the daily dose exceeded 40 mg/kg (143). At a daily dose of 250 mg/kg during pregnancy TMTD was teratogenic in hamsters. Likewise, TMTD dose-dependently increased fetal mortality (130). TMTD also increased the incidence of tibial dyschondroplasia in chickens (30). When tested in eggs TMTD increased dose-dependently fetal mortality and the incidence of malformed fetuses as well (81). Based on all available data TMTD probably is a weak but a true teratogen and an embryotoxic agent.

Ziram. There are no data of the embryotoxic or teratogenic effects of ziram in humans. Daily doses of 50-100 mg/kg of ziram during pregnancy reduced fetal weights in rats. Maternal toxicity was evident at all fetotoxic dose levels (47). The lowest dose, 25 mg of ziram/kg daily, was without an effect in pups and dams. In mice, ziram at daily doses of 100-200 mg/kg by gavage caused infertility, and increased fetal mortality during pregnancy. It also increased the incidence of skeletal malformations such as cyphosis, scoliosis, failure of sternal ossification, and caused retardation of skeletal development (14).

10 RELATION BETWEEN EXPOSURE, EFFECT AND RESPONSE

It is difficult to obtain an exact conception of the dose-effect and dose-response relationships in humans for thiurams and DMDC's because the human data of most of the compounds are so scanty. Long-term effects in humans are virtually unknown. Moreover, in occupational environment the exposure to thiurams or DMDC's occurs mainly via ambient air or via skin, and there is almost a complete absence of toxicity studies in humans or experimental

Table 2. Summary of effects of disulfiram (DSF) or diethyldithiocarbamate (DDC) on experimental animals and man.

Daily Exposure	Species	Route	Effect	Reference
100 mg/kg of DSF for 6 weeks	rat	i.p.	damage of cortical neurons	155
330 mg/kg of DDC for 4-9 weeks	rabbit	i.p.	degenerative changes in medulla, cerebellum and spinal cord	29, 129
1000 mg of DSF	man	oral	decrease of serum TSH and prolactin levels	11
25 mg/kg of DSF for 30 days	rat	oral	thyroid hyperplasia and increased thyroid weight	150

animals applying these exposure routes, cutaneous hypersensitivity studies included. Mainly effects on the nervous system and genotoxicity have been studied. In the following, thiurams and DMDC's are considered as one group of compounds. However, tables 2-5 show exposure-effect relationships for some critical effects for each compound separately.

The signs of long-term exposure to thiurams and DMDC's are non-specific. Experimental animals show fatigue, hair loss, reduced weight gain and shortened life span. All these effects are related to the dose (see 41, 61). At higher dose levels during long-term exposure, neurological signs such as paralysis of hind legs (61) are a common finding. Repeated doses of some thiurams lead to hyperplasia of the thyroid gland (150), presumably because of the inhibition of the synthesis of thyroid hormones which leads to decreased serum thyroxin and increased serum TSH levels, possibly due to the inhibition of dopamine-ß-hydroxylase

and subsequent disturbances in brain monoamine synthesis (11, 82, 83). Lowy and coworkers (91, 92) have made detailed 30-day dose-effect studies at doses of 11-60 mg of TMTD/kg daily with rats to find out the most sensitive and reliable parameter predicting the toxic actions of thiurams and DMDC's. They found that the absolute weight of the body and several tissues, especially seminal vesicles and testes, liver protein labelling, and the adjusted weights of the liver and testes were the most sensitive indicators of TMTD toxicity when log-probit model was applied for the analysis of the data. This is in agreement with the findings that gonads are sensitive targets of some of these compounds. No data are available to evaluate the effects of ambient concentrations of these agents.

Table 3. Summary of long-term effects of ferbam in experimental animals.

Exposure	Species	Route	Effect	References
125 mg/kg daily for 2 years	rat	oral	cystic brain lesions, convulsions, paralysis of hind legs	60, 61
96 mg/kg, 80 weeks	rat	oral	ataxia, hyperactivity, convulsions, thyroid metaplasia	85
25 mg/kg daily for 1 year	dog	oral	clonic convulsions	61

All the compounds, except DSF, are strong allergens. Moreover, sensitization to one of the compounds easily leads to cross-allergy towards the other members of the same class.

Thiurams and DMDC's are also appreciable mutagens in several test systems with and without metabolic activation. Mutagenicity increases in a dose-dependent fashion. Also in workers occupatio-

nally exposed to ziram the number of leukocytes with chromosomal

Table 4. Summary of the long-term effects of thiram on experimental animals.

Exposure	Species	Route	Effect	References
96 mg/kg daily for 80 weeks	rat	oral	liver enlargement	85
52-67 mg/kg for 80 weeks	rat	oral	ataxia, paralysis of hind legs, degeneration of motorneurons	84
52-67 mg/kg for 80 weeks	rat	oral	thyroid metaplasia	85
132 mg/kg daily for 13 weeks	rat	oral	tubular degeneration of the testes, atypical spermatids, infertility	85
250 mg/kg daily during pregnancy	hamster	oral	increased fetal mortality, teratogenicity	130
30 mg/kg	mouse	i.p.	chromosomal aberrations and number of abnormal sperms	122, 168
500 mg/kg, single dose	mouse	i.p.	increased incidence of micronuclei in polychromatic erythrocytes in bone marrow	116

aberration was considerably increased. Contrary to this, none of the compounds has proved a carcinogen. There is only one study providing evidence of the carcinogenic effects of ziram in rats and mice (64).

Table 5. Summary of long-term effects of ziram in experimental animals and man.

Exposure	Species	Route	Effect	References
250 mg/m^3 for 4 hours 1-4 times	guinea pigs	lungs	respiratory distress, hemorrhage in the lungs, death	99
50 mg/m^3 for 4 hours	mouse rat	lung lung	LC_{50}-value LC_{50}-value	99
15 mg/m^3 for 4 hours	rabbit	lung	inflammation of the respiratory tract, interstitial pneumonia, LC_{50}-value	35, 99
125 mg/kg daily for 2 years	rat	oral	behavioral alterations, ataxia, paralysis of hind legs	61
25 mg/kg daily for 1 year	dog	oral	convulsions, death	61
200 mg/kg during pregnancy	mouse	oral gavage	infertility, increased fetal mortality	14
30 mg/kg daily for 2 years	rat	oral	C-cell carcinomas of the thyroid	64
occupational exposure	man	lung	increased number of chromosomal aberrations in peripheral leucocytes	121

11 NEEDS FOR FURTHER RESEARCH

There is a need to conduct short- and long-term inhalation studies to study the toxic effects of thiurams and DMDC's, especially on thyroid and pituitary function, liver, gonadal function and

morphology. Dose-effect and dose-response relationships for critical target organs should be clarified for each compound separately. Detailed pharmacokinetic studies are important, and they should be combined with analytical studies. Moreover, biological exposure indicators, such as inhibition of erythrocyte aldehyde dehydrogenase, should be sought for, and applied for the monitoring of the exposure of workers in relevant occupational environments.

12 DISCUSSION AND EVALUATION

Thiurams and DMDC's are fungicides and accelerators in the rubber industry. There are only few reports of their toxicity in humans. Most information is derived from animal and in vitro experiments.

Disulfiram. The acute toxicity of DSF is low and directed mainly towards the nervous system as well as the thyroid gland. High doses of DSF may damage the liver. DSF is not mutagenic; data on the carcinogenicity of DSF is inadequate. It is not appreciably irritating or sensitizing. The critical effects of DSF are directed towards the thyroid gland.

Ferbam. Both the short- and long-term effects of ferbam are directed towards thyroid and nervous system. Ferbam is mutagenic but data on its carcinogenicity is inadequate. Ferbam is not considerably irritating. Sensitization and cross-sensitization with the other thiurams are the critical effects of ferbam.

Thiram. Thiram has toxic effects on nervous system, and long-term exposure to thiram may cause thyroid metaplasia. Thiram is teratogenic and embryotoxic, and it is clearly mutagenic in bacterial tests, and causes sperm anomalies. Evidence on the carcinogenicity of thiram is inadequate. The critical effects of thiram are strong sensitization and cross-sensitization with other thiurams.

Ziram. Ziram causes respiratory distress and chromosome aberrations in exposed individuals. In animals, ziram causes toxic effects in the nervous system. It is embryotoxic and mutagenic in bacterial test systems. Evidence on the carcinogenicity of ziram is inadequate. The critical effects of ziram are its strong sensitizing properties and cross-sensitization with other thiurams.

In discussions of occupational exposure limits for thiurams and DMDC's primary consideration should be given to their effects on thyroid and gonads, and sensitizing properties.

13 SUMMARY

K. Savolainen. 89. Thiurams and dimethyldithiocarbamates. Nordic expert group for documentation of occupational exposure limits. Arbete och Hälsa 1990:--, pp.----.

A critical survey of the literature relevant for the discussion of an occupational exposure limit is given.

Disulfiram. The critical target of the toxic effects of DSF is the thyroid gland.

Ferbam. The critical effects of ferbam are sensitization and cross-sensitization with other thiurams.

Thiram. The critical effects of thiram are sensitization and cross-sensitization with other thiurams. Thiram may also affect spermatogenesis.

Ziram. The critical effects of ziram are sensitization and cross-sensitization with other thiurams.

The present occupational exposure limit values for thiurams and DMDC's are mainly based on their sensitizing properties and effects on skin. There is a need to conduct short- and long-term inhalation exposure studies to clarify dose-effect relationships

between effects and exposure to thiurams and DMDC's in thyroids and gonads.

Key words: Disulfiram, ferbam, metabolism, occupational exposure limit value, organ effects, reproduction toxicity, sensitization, thyroid gland, thiram, ziram.

A Swedish version is available in Arbete och Hälsa, 1990:--. 171 references.

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C. **Thiram.**

Country	mg/m³	Year	Note	Ref
BRD	5	1988	total dust	5
Denmark	2	1988	-	2
Finland	5	1987	S	11
	10		15 min	
France	5	1988	-	12
Great Britain	5	1988	-	4
	10		STV	
Iceland	5	1978	-	9
The Netherlands	5	1989	-	7
Norway	5	1984	A	1
Soviet Union	0.5	1978	-	6
USA (ACGIH)	5	1988-1989	-	10
(OSHA)	5	1989		8

A = Allergen
S = Skin
STV = Short-term value

D. **Ziram.**

Country	mg/m³	Year	Note	Ref
BRD	0.1	1988	S	5
Denmark	-	1988	-	2
Finland	-	1987	-	11
France	-	1988	-	12
Great Britain	-	1988	-	4
Iceland	-	1978	-	9
The Netherlands	-	1989	-	7
Norway	-	1984	-	1
Soviet Union	-	1978	-	6
Sweden	-	1988	-	3
USA (ACGIH)	-	1988-1989	-	10
USA (OSHA)	-	1989	-	8

S = Skin

APPENDIX I. **Occupational exposure limits for airborne thiurams and diethyldithiocarbamates.**

A. **Disulfiram**

Country	mg/m³	Year	Note	Ref
BRD	2	1988	-	5
Denmark	-	1988	-	2
Finland	2	1987	-	11
	6		15 min	
France	2	1988	-	12
Great Britain	-	1988	-	4
Iceland	-	1978	-	9
The Netherlands	2	1989	-	7
Norway	-	1989	-	1
Soviet Union	-	1978	-	6
Sweden	-	1988	-	3
USA (ACGIH)	2	1988-1989	-	10
USA (OSHA)	2	1989	-	8

B. **Ferbam**

Country	mg/m³	Year	Note	Ref
BRD	15	1988	total dust	5
Denmark	5	1988	-	2
France	10	1988	-	12
Great Britain	10	1988	-	4
	20		STV	
Iceland	5	1978	-	9
The Netherlands	10	1989	-	7
Norway	5	1984	-	1
USA (ACGIH)	10	1988-1989	-	10
(OSHA)	10	1989	total dust	8
	5	1989	inhaled fraction	8

STV = Short-term value

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N-NITROSO COMPOUNDS AND CANCER

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CONTENT

1. ABBREVIATIONS

ENU	N-nitrosoethylurea
HNEU	N-nitrosohydroxyethylurea
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
MNU	N-nitrosomethylurea
NAB	N-nitrosoanabasine
NDBA	N-nitrosodibutylamine
NDCA	N-nitrosododecylamine
NDEA	N-nitrosodiethylamine
NDELA	N-nitrosodiethanolamine
NDMA	N-nitrosodimethylamine
NDPA	N-nitrosodipropylamine
NDPhA	N-nitrosodiphenylamine
NEMA	N-nitrosoethylmethylnitrosamine
NG	N-nitrosoguvacoline
NHPYR	N-nitrosohydroxypyrrolidine
NMBA	N-nitroso-methylbenzylamine
NMDA	N-nitroso-N-methyldodecylamine
NMOR	N-nitrosomorpholine
NMPhA	N-nitrosomethylphenylamine
NMTA	N-nitroso-N-methyltetradecylamine
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N-nitrosonornicotine
NOC	N-nitroso compounds
NOCA	N-nitrosooxazolidine-4-carboxylic acid
NPIP	N-nitrosopiperidine
NPRO	N-nitrosoproline
NPYR	N-nitrosopyrrolidine
NTCA	N-nitrosothiazolidine-4-carboxylic acid
NTHZ	N-nitrosothiazolidine
NVNA	Non-volatile nitrosamines
TSNA	Tobacco-specific nitrosamines
VNA	Volatile nitrosamines

2. INTRODUCTION

In 1937 Freund reported the hepatotoxic effect of N-nitroso-dimethylamine (NDMA) describing findings in two laboratory technicians intoxicated by NDMA (47). Experimental investigations of the biological effects of NDMA and related compounds were started after the initial discovery in 1956 when Magee and Barnes reported on the hepatotoxicity and carcinogenicity of NDMA in laboratory animals (89). Liver disease observed in mink and ruminants in Norway during the years 1957-1962 stimulated further research in this area. These studies showed that the liver disease was related to a herring preserved in sodium nitrite that was used to feed the animals. The substance was isolated and identified as NDMA (32,33). Methylamines occur naturally in fish and could react with nitrite to form nitrosamines. Important reports were also given by Schoental on the carcinogenicity of nitrosamides (128,130-132), and later a report was given by Druckrey et al in 1967 in which they described the organotropic carcinogenic effects of 65 N-nitroso compounds in rats (25).

The potent carcinogenicity of most N-nitroso compounds in experimental animals has led to considerable attention, and N-nitroso compounds have been extensively studied in many laboratories. The number of scientific papers relating to the studies of N-nitroso compounds are numerous and a number of excellent reviews have appeared on N-nitroso compounds since the initial report by Magee and Barnes. Only the major findings will be summarized here. The question considered in this review are problems associated with relevance of N-nitroso compounds to human cancer. Most relevant to the topic here is the formation and exposure to N-nitroso compounds in occupational situations and the potential occupational cancer-risk these chemical agents represent.

3. CHEMICAL AND PHYSICAL DATA

The chemistry of N-nitroso compounds have been reviewed (see 38). The physico-chemical data of several N-nitroso compounds are presented in table 1. This class of compounds fall into two groups, the nitrosamines and the nitrosamides. The nitrosamides are direct acting, whereas the indirect acting nitrosamines require metabolic activation.

Table 1 Chemical formulas, boiling points, melting points, molecular weights and CAS numbers of some N-nitroso compounds

Compound	Formula	Boiling point(^{0}C)	Melting point(^{0}C)	Molecular weight	CAS no.
ENU	$C_3H_7N_3O_2$		102-104	117.13	759-73-9
HNEU	$C_3H_7N_3O_3$		51-55	133.11	13743-07-2
MNNG	$C_2H_5N_5O_3$		118	147.12	70-25-7
MNU	$C_2H_5N_3O_2$		124	103.1	684-93-5
NAB	$C_{10}H_{13}N_3O$	170-180		191.23	1133-64-8
NDBA	$C_8H_{18}N_2O$	105		158.24	924-16-3
NDEA	$C_4H_{10}N_2O$	177		102.1	55-18-5
NDELA	$C_4H_{10}N_2O_3$	114 (1.5mmHg)		134.1	1116-54-7
NDMA	$C_2H_6N_2O$	151		74.1	62-75-9
NDPA	$C_6H_{14}N_2O$	81 (5mmHg)		130.2	621-64-7
NDPhA	$C_{12}H_{10}N_2O$		66.5	198.2	86-30-6
NEMA	$C_3H_8N_2O$	163		88.1	10595-95-6
NG	$C_3H_5N_3O_9$	137		170.17	55557-02-3
NMOR	$C_4H_{10}N_2O_2$	224-225		116.1	59-89-2
NMDA	$C_{13}H_{28}N_2O$	136-138		228.38	55090-44-3
NMPhA	$C_7H_8N_2O$	225		136.15	614-00-6
NNK	$C_{10}H_{13}N_3O_2$		63-65	207.2	64091-91-4
NNK	$C_9H_{11}N_3O$	154		178	53759-22-1
NOBA	$C_8H_{18}N_2O$	116		158.2	924-16-3
NPIP	$C_2H_{10}N_2O$	215		114.2	100-75-4
NPRO	$C_5H_8N_2O_3$		100-101	144.0	7519-36-0
NPYR	$C_4H_8N_2O$	214		100.14	930-55-2

The nitrosamines have the general structure (fig 1):

Fig. 1

R_1 and R_2 can be many different organic groups. The R_1R_2N can come from a primary, secondary, or tertiary amine. The nitrosyl group, NO, can come from nitrogen oxides (NO, NO_2, N_2O_4 or N_2O_3) or nitrite.

For example, the alkyl-N-nitrosamine have R_1 and R_2 as alkylgroups, the N-nitroso-amino acid have R_1 or R_2 as a carboxylic acid; the nitrosamides have R_1 as an alkyl group and R_2 may be an aminogroup (N-nitrosourea).

Nitrites react with amines to produce nitrosamines and with amides to produce nitrosamides. The reaction with nitrite is called nitrosation as shown in fig 2:

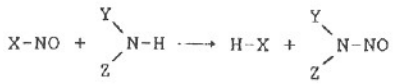
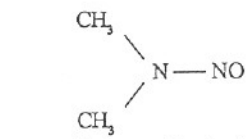


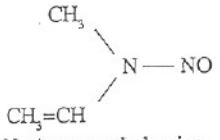
Fig. 2

N-nitrosation can occur at either acidic, neutral or alkaline conditions depending on the reactans and the catalysts present. Generally, N-alkylureas, N-arylureas, N-alkyl-carbamates, secondary aromatic amines, secondary amine derivatives of piperazine and morpholine are more readily nitrosated than simple aliphatic secondary and tertiary amines. However, the kinetics are modified by catalysts (96). Furthermore, nitrosamines undergo photolytic decomposition. Exposure to ultra-violet light splits off the nitrosogroup yielding nitrite (16,129).

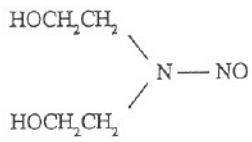
The structure of some N-nitroso compounds are shown in Fig 3.



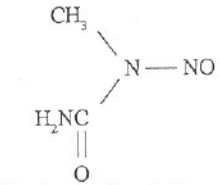
N-nitrosodimethylamine



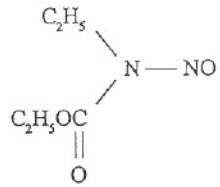
N-nitrosomethylamine



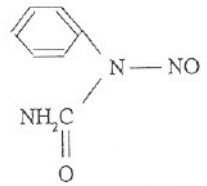
N-nitrosodiethanolamine



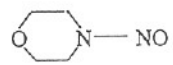
N-nitroso-N-methylurea



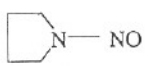
N-ethyl-N-nitrosourethane



N-nitrosophenylurea



N-nitrosomorpholine



N-nitrosoproline

Fig 3 Structures of some N-nitroso compounds

4. OCCURRENCE AND EXPOSURE

Because of their potent carcinogenic activity, wide occurrence, and possible *in vivo* formation from precursor amines and nitrosating agents, considerable efforts have been made to assess human exposure to N-nitroso compounds. Human exposure to N-nitroso compounds can be divided into uptake of preformed N-nitroso compounds from occupational exposure and environmental sources, and endogenous exposure (table 2). Nitrosamines have been detected in processed meat, tobacco and its smoke, in some alcoholic beverages, agricultural chemicals, cosmetics, in urban air, in drinking water, and in certain occupational settings. Thus, this class of compounds represent an important class of chemical carcinogens.

Table 2 Total human exposure to N-nitroso compound

Preformed NOC	Endogenous nitrosation
Work environment	Nitrosating agents
Leather industry	Nitrite
Metal working industry	Nitrous gases
Rubber industry	Nitrite from nitrate
Chemical industry	Precursor amines
Foundries	
Pesticide production	
Fish processing	
Detergent production	
Environmental	
Food	
Tobacco	
Cosmetics	
Drugs	
Indoor air pollution	
Household-commodities	

4.1 Analysis of N-nitroso compounds

Most nitrosamines can be determined without difficulty at very low levels by gas and liquid chromatography and thermal energy analyser (TEA)(41). Mass spectrometry is available for the characterization of unknown nitrosamines and a technique has been described involving laser for the detection of NO-containing molecules (93). There is, however, a lack of adequate analytical techniques for nonvolatile N-nitroso compounds.

4.2 Environmental exposure

4.2.1 Air

Nitrosamines are detected in the air. NDMA occured in concentrations of 3-320 ngm^{-3} in airsamples from Baltimore in Maryland and 5-170 ngm^{-3} in the air from Belle in West-Virginia. This amount of NDMA is relatively large. In comparison, benzo(a)pyrene occurs in concentrations of 2ngm^{-3} in urban air. N-nitroso compounds were also detected in airborn particles (41-44). Nitrosamines were not detected in airsamples from Boston, New Jersey and various places in the UK (156). In a polluted area in Austria, the concentration of N-nitroso compounds were between 10 and 40 ngm^{-3} (140,141). A significant contribution to the nitrosamine burden is exposure via nitrogen oxide in air.

4.2.2 Tobacco smoke and tobacco products.

About 50 compounds present in tobacco smoke and products have been shown to be carcinogenic in experimental animals (including tobacco-specific (TSNA), volatile (VNA) and nonvolatile (NVNA) N-nitrosamines). Table 3 lists some of the major nitrosamines that are found in both phases.

Table 3 Nitrosamines in tobacco smoke

N-Nitrosodimethylamine	N-Nitrosodiethanolamine
N-Nitrosoethylmethylamine	N'-Nitrosonornicotine
N-Nitrosodiethylamine	4-(N-Nitrosomethylamino-1-(3-pyridyl)-1-butanone
N-Nitroso-di-n-propylamine	N'-Nitrosoanatabine
N-Nitroso-di-n-butylamine	N'-Nitrosoanabasine
N-Nitrosopyrrolidine	
N-Nitrosopiperidine	

The TSNA are the most abundant strong carcinogens in chewing tobacco, snuff, tobacco-containing betel quid, and tobacco smoke (table 4-6) (11,146). N-nitrosonornicotin (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-(butanone) (NNK) are quantitatively the major known carcinogens present in unburned tobacco. NNK is the most potent tobacco-specific carcinogenic nitrosamine known, inducing lung tumors in all species tested.

Table 4 Carcinogenicity and target organs of TSNA in different species.

TSNA	Route of admin.	Experimental animal	Cancer site	ref.
NNK	s.c.	rat	Nasal cavity, lung, liver	74,11
NNK	i.p.	hamster	Nasal cavity, trachea, lung	
NNN	oral	rat,	Nasal cavity, esophagus	
		hamster	Nasal cavity	
NNN	s.c.	rat	Nasal cavity	
		hamster	Trachea	
NNN	i.p.	hamster	Nasal cavity	
		mouse	Lung	

Table 5 Tumorigenic N-nitrosamines in tobacco and tobacco smoke

Compounds	Tobacco (per gram)	Mainstream (per cigarette)	Evidence from IARC-evaluation of carcinogenecity in lab animals	ref.
NDMA	ND- 215 ng	0.1-180 ng	sufficient	74
NEMA		3 - 13 ng	sufficient	
NDEA		ND- 25 ng	sufficient	
NPYR	ND- 260 ng	1.5-110 ng	sufficient	
NDELA	ND-6900 ng	ND - 36 ng	sufficient	
NNN	0.3-89 µg	0.12-3.7 µg	sufficient	
NNK	0.2- 7 µg	0.08-0.77 µg	sufficient	
NAB	0.01-1.9 µg	0.14-4.6 µg	limited	
NMOR	ND-690 ng		sufficient	

Table 6 Concentrations of N-nitrosamines in sidestream smoke of cigarettes (ng/tobacco-product)[A]

Tobacco product[B]	Volatile N-nitrosamines[C]			Tobacco specific nitrosamines[C]		
	NDMA	NEMA	NPYR	NNN	NNK	NAT
US non-filter cigarette(1)	680(52)	9.4(5)	300(27)	1700(7)	410(4)	270(0.8)
US filter cigarette(1)	736(139)	10(8)	387(76)	150(0.5)	190(1.3)	150(0.4)
French non-filter cigarette(1)	823(19)	30(25)	204(9)			
French filter cigarette(1)	1040(160)	10(20)	213(25)			

[A] Adapted from ref. 74
[B] In parentheses, number of cigarettes used
[C] In parentheses, ratio of content in sidestream smoke to that in mainstream smoke

These results suggest a possible role of NNK in the induction of lung cancer. Analytical studies have documented the levels in tobacco and tobacco-smoke. The level of NNN and NNK in chewing tobacco, snuff, and cigarette smoke are at least two orders of magnitude higher than the concentrations of N-nitrosamines in other consumer products or respiratory environment.

There is a much higher level of volatile N-nitrosamines in sidestream smoke compared to mainstream smoke (table 6). Mixtures of betel quid and tobacco also contain NNN (0.025-0.1 ppm), NNK and NG (<0.014 ppm). The mainstream smoke of an American filter cigarette contains about 310 ng of NNN and 150 ng of NNK (65,69,97,154).

VNA in tobacco smoke are formed by the reaction between amines and nitrogen oxides during the combustion process. Only a relatively small amount originates from preformed VNA present in cured tobacco. The most abundant VNA in mainstream are NDMA and N-nitrosopyrrolidine (NPYR) (70). N-nitrosoproline (NPRO) found in unburned tobacco can serve as a precursor to NPYR via decarboxylation during the combustion process (13). The non-volatile N-nitroso amino acids found in tobacco may decarboxylate, giving rise to the carcinogenic nitrosamines N-nitrosoethylmethylnitrosamine (NEMA) and N-nitrosopiperidine (NPIP)(105).

4.2.3. Food

Studies have demonstrated a relatively high content of precursors of N-nitrosamines (nitrite, nitrate, and secondary amines) as well as some preformed N-nitrosamines present in various foods (animal products, vegetable products and beverages).

Meat products preserved with nitrite and nitrate contain $\mu g kg^{-1}$ amounts of N-nitroso compounds (table 7 and 8, see appendix II). However, improved production technologies, reduced levels of nitrate and nitrite and the addition of ascorbic acid and α-tocopherol (nitrosation inhibitors) have resulted in reductions in the levels of volatile nitrosamines.

N-nitroso compounds may readily form in fish, as they are rich in amino compounds, mainly trimethylamine oxide, trimethylamine, and dimethylamine. Thus, analysis of nitrosamines in fish and fish products (both fresh and processed) have shown that fish contains nitrosamines, mainly NDMA (151) (table 9, see appendix II). Generally, it can be concluded that NDMA concentration in fish and seafood is low, although very little data are available for nonvolatile nitrosamines in fish. However, there are some discrepancies in the literature. Thus, relatively high levels of NDMA were found in salted fish, commonly used in China.

NDMA has also been found in dairy products (table 10, see appendix II). Furthermore, volatile N-nitroso compounds have been detected in a variety of fermented vegetables, grain products (table 11, see appendix II) (151) and in samples of beverages including brandy and whisky (Table 12 , see appendix II).

4.2.4 Cosmetics

Fan et al reported that N-nitrosodiethanolamine (NDELA) was present in some cosmetics such as lotions and shampoos in amounts ranging from less than 1 to 48.000 µg/kg (table 13) (151).

Table 13 Nitrosamines in cosmetics (µg/kg).

	NDELA	NMOR	NMDA	NMTA	Ref.
Cosmetics					
American	<48000				36
French	20-4113				82
Hair-care products with lauramine oxide			11-873	8-254	66
Cosmetics and toiletries	45-1400	80-640	1.5-24		139

4.2.5 Pesticides and herbicides

Nitrosamine contamination of pesticides can occur. It is mainly confined to dinitroaniline herbicides, dimethylamine salts of phenoxyalkanoic acid herbicides, diethanolamine and triethanolamine salts of acid pesticides, quarternary ammonium components and morpholine derivatives (table 14).

Table 14 Nitrosamines in pesticides(mg/kg)

	NDMA	Ref.
Phenoxyalkanoic acid pesticides	0.1-8,5	122
Ammonium chloride pesticides	4.8-168	122

4.2.6 Water

Well water may contain nitrates and trace levels of NDMA and NDEA (<0.01 µg/l) (98). Industrial waste water and sewage sludge may contain NDMA, NDEA and other nitrosoamines in concentrations up to 5µg/l (59).

4.3. Occupational exposure to N-nitroso compounds

Human exposure to N-nitroso compounds is highest in the industry. In industries such as rubber production and processing, manufacture and use of machining and grinding fluids, and in leather tanning relatively high levels of N-nitroso compounds have been found.

4.3.1 Rubber industry

Studies have shown that workers in the rubber industry have significantly increased incidence of cancer (stomach, intestine, lung, bladder, brain, lymphatic and haematopoietic systems)(73). The exposure to N-nitroso compounds is believed to be one of the possible causes of occupational cancer in this workplace. Air monitoring of rubber plants showed a widespread occurrence of considerable levels of carcinogenic nitrosamines (Table 15) (151).

Table 15 Rubber industry ($\mu g/m^3$)

	NDMA	NMOR	NPYR	NDEA	NDPhA	Ref.
Workroom air	<1	<250	<1	<1	<1230	123
Process samples tyre industry	<1050	<4700		<210		100
Hot process area	0.05-2	0.1-17				138
Tube production workroom air	130[1] 40[2]					138
Injection moulding and curing of conveyer belts	1600[3] 90[4]	4700[3] 380[4]				138

1 Use of NDPhA as retarder and tetramethylthiuram disulphide as accelerator
2 Vulcanisator process using tetramethylthiuram disulphide as an accelerator
3 Process samples
4 Personal monitoring

The nitrosamines NDMA, NDEA, NDBA, NPIP, NPYR, NMOR and NDPhA have been identified in workplace air. The highest levels of N-nitroso compounds measured in the atmosphere are found in the rubber industry.

4.3.2 Leather tanning industry

Dimethylamine, a precursor of NDMA, is used as a depilatory agent in some tanneries. The largest daily exposure to NDMA occurs in the leather industry. NDMA was detected in the atmosphere of a leather tannery at levels up to 47 $\mu g/m^3$ (Table 16). However, after cleaning of the plant the level of NDMA decreased to 0.1-3.4 $\mu g/m^3$. The highest levels were in the retanning and fatliquoring area (151).

Table 16 Leather tanning industry ($\mu g/m^3$)

	NDMA	NMOR	Ref.
Nine different tanneries	0.05-47 (mean 3.4)	0.05-2.0 (mean 0.2)	35
one tannery	<47		124

4.3.3 Metal working industries

Synthetic metal working fluids often contain nitrosamines. Analysis have shown that NDELA is present as an impurity in cutting oils used in metal working industries (table 17) (151). NDELA are formed from ethanolamines and nitrite in the grinding fluids. Up to 3% NDELA has been detected (37,142, 160). NDELA has been found in several brands of antifreeze (22). NDELA can readily be formed by nitrosation of di- or tri-ethanolamine. NDELA can penetrate the skin and thereby increase the risk of exposure to this carcinogen (28,119).

4.3.4 Other industries

Studies have demonstrated small quantities of NDMA and NDEA in factories producing amines and pharmaceuticals. NDMA were also found in foundries and in processing and preservation of fish (table 17).

Table 17 Other industries

Industry	NDMA $\mu g/m^3$	NDELA mg/g	NDEA $\mu g/m^3$	Ref.
Cutting fluids		0.2-29.9		37
(di-and triethanolamine based)		0.4-4.2		160
Antifreeze		0.1		22
1 foundry	0.1		0.02-1.4	35
8 foundries	<0.35			21
Fish processing	0.01-0.06			35
1,1-dimethylhydrazine producing	6-36			43
Methylamine producing	0.01-1			39
Surfactant and detergent producing	0.03-0.8			35
Dye manufacturing	0.03-0.1		0.03-0.06	35

4.3.5 Passive smoking

Many known carcinogens have been identified in tobacco smoke (see 4.2.2). Sidestream smoke, which is formed in between puff-drawing, may differ quantitatively from the mainstream smoke which is inhaled only by the active smoker. Some compounds occur in higher concentrations in sidestream smoke. Although this smoke is diluted by the ambient air, the

passive smoker may inhale smoke which is qualitatively richer in certain compounds than mainstream smoke. There is a 6-100 times larger release of carcinogenic volatile N-nitrosamines into sidestream compared to mainstream smoke (10). Such differences make it difficult to predict the biological effect of exposure to sidestream smoke. Generally, levels of N-nitrosamines and PAH exceed maximum levels reported for urban air pollutants by one to three orders of magnitude (table 18) (12).

Table 18 Concentrations of nitrogenoxides and N-nitrosamine pollutants in various indoor spaces ($\mu g/m^3$)

Pollutant	Location	Concentration	Ref.
Nitrogen oxide	workrooms	39-345	157
Nitrogen dioxide	workrooms	50	157
NDMA	public places	0.001-0.24	12,143
NDEA	public places	<0.01-0.2	143

5. METABOLISM AND DNA ADDUCT FORMATION

5.1 Pharmacokinetics

Animal studies show that the route of administration appears to play a role in determining the distribution of N-nitrosamines in the organism. In humans, absorption of N-nitrosamines into the body probably occurs mainly by inhalation. Studies in rat indicate little absorption of NDMA from the stomach and a rapid absorption from the small intestine (60). NDMA is then transferred to the liver. On the other hand, after i.v. exposure to NDMA, a more uniform distribution is observed (77). Studies indicate that low doses of NDMA (<1 mg/kg) are metabolized extensively by the liver (107). In the rat, NDELA is excreted largely unchanged in the urine (120).

5.2 Biotransformation

Nitrosamine carcinogenesis depends on the distribution of the compounds in the organism, the metabolic capacity of organs, the specificity of DNA damage and the extent and precisions of DNA repair. The chemically reactive N-nitrosamides (e.g. N-nitroso-N-alkylureas) decompose by hydrolysis. The rate of decomposition is greatly increased by the presence of SH-compounds such as cysteine. The chemically stable N-nitrosamines, like NDMA, require metabolic activation to form the same alkylating electrophiles. The major activation pathway is believed to be the oxygenation of the α-carbon (a reaction generally known as α-hydroxylation) and subsequent dealkylation of these reactive intermediates to alkyldiazohydroxides and finally alkylating agents, which subsequently react with cellular macromolecules (nucleophilic sites), including DNA, forming stable DNA-adducts (23,90, see chapter 5.4). Besides the alkyldiazonium ions, alkyldiazohydroxides are also good candidates as ultimate carcinogens. Following metabolic activation, N-nitroso compounds will in addition to alkylcarbonium ions also give rise to nitrogen and aldehydes (Fig 4). Formaldehyde is mutagenic in some bacteria, fungi, Drosophila and cultured human cells (3,52). Formaldehyde produces nasal carcinoma in rats (145). Much of the information of N-nitrosamine metabolism *in vivo* is based on the studies with the volatile alkylnitrosamines, particularly NDMA and NDEA.

The involvement of cytochrome P450 in the metabolism of N-nitroso compounds has been demonstrated, however the level of importance of the P450 dependent monooxygenase has been questioned. There is also strong evidence implicating other enzymes in the activation process. The longer chain dialkyl nitrosamines can undergo β-hydroxylation to methylalkyl nitrosamines prior to α-hydroxylation (84,106). Metabolism of cyclic nitrosamines such as N-nitrosopyrrolidine also appear to occur via a number of pathways (64). Compared to other

tissues, liver exhibits a greater ability for metabolizing N-nitroso compounds. However, there is considerable evidence that extrahepatic tissue can metabolize a wide range of N-nitrosamines, thereby converting them to forms which bind to DNA (table 19) (8).

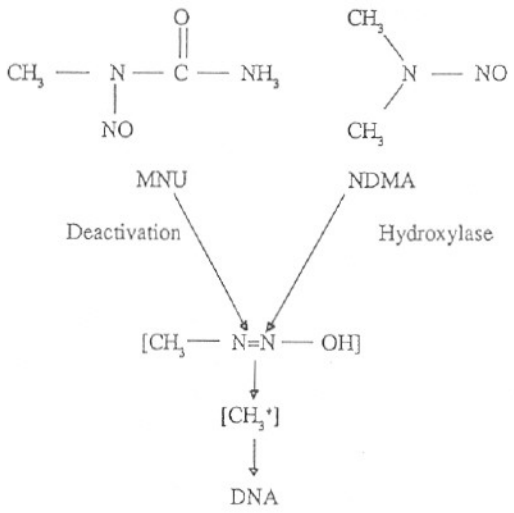


Fig. 4 A simplified scheme of the metabolism of MNU and NDMA

Table 19 N-nitrosoamines activated to form metabolites associated with DNA by cultured adult human tissues and cells

N-nitroso compound	Bronchus	Colon	Esophagus	Bladder	Ref.
Dimethylamine	+[A]	+	+	+	4,5,56,58
Diethylamine	+	±	+		4,6,57
Pyrrolidine	+	+	-	+	4,5,6,57,58
Piperazine	+	±	-		4,57
Methylbenzyl-amine			-		6

[A] (+) positive; (-) nondetectable; (±) metabolized to a small extent or not at all.

5.3 Endogenous N-nitrosation

Humans are also exposed to carcinogenic N-nitroso compounds from endogenous synthesis, which may represent a major source of human exposure. In vivo formation of carcinogenic N-nitroso compounds was first indicated by Sander and Bürkle (125). Ohshima and Bartsch provided the first conclusive demonstration that nitrosamines can be formed endogenously in man (101). Endogenous nitrosation in humans has been estimated by measuring N-nitrosoproline (NPRO) excretion in urine after ingestion of nitrate or proline: A human subject, fasted overnight, is given nitrate. After 30 minutes the subject ingests the secondary amino acid L-proline. Urine is collected and analyzed by gas chromatography for the NPRO. The formation of NPRO is proportional to the L-proline dose and to the square of the nitrate dose.

The human diet contains a variety of nitrosatable amino precursors. However, several factors influence the endogenous formation of N-nitroso compounds (table 20).

Table 20 Factors influencing the endogenous formation of N-nitrosation

Type and quantity of amine/amide precursors in the diet
Levels of dietary nitrate
Atmospheric NO exposure
Salivary nitrate reductase activity
Gastric pH
Bacteria in the stomach
Presence of nitrosation catalysts/inhibitors in the diet

There is evidence that some of the nitrite could be converted to nitrosamines in vivo. Bacteria- and acid-catalyzed nitrosation has been clearly demonstrated (55,68,95,111). In a wide range of mammalian species, but not in healthy humans, it has been shown that considerable amounts of microorganisms

can excist in the stomach and it seems clear that microflora may play a role in gastric tumorigenesis. Eighty percent of nitrite entering the stomach is produced by bacterial reduction of nitrate from the diet. The remaining 20% of nitrite entering the stomach arise from nitrite consumed in the diet. Most *in vivo* nitrosation occurs probably in the stomach, where acid catalyzes the reaction. Furthermore, studies have shown that nitrogen oxide can nitrosate amines in the lung. Since nitrogen oxides and other nitrosating agents are present in tobacco smoke, smokers may be exposed to higher levels of endogenously formed N-nitroso compounds than non-smokers. Nitrogen oxides occur also in polluted air.

5.4 DNA-adduct formation

DNA-carcinogen adducts in target cells are considered most likely to initiate genetic changes which eventually may lead to malignant neoplasia. A number of nitroso-compound-DNA-adducts have been reported in the literature such as O^6-alkylguanine, O^2-and O^4-alkyl-thymidine, 1-,3- and 7-methyladenine, 7-methylguanine, and O^2-ethylcytosine (Fig 5). Of these O^6-alkylguanine and O^4-alkylthymidine are believed to be the major contributors to the mutagenicity and carcinogenicity of these agents (51,83,144). O^6-alkyl-guanine:thymine and O^4-alkylthymine:guanine mispairs have been reported both *in vivo* and *in vitro* to mediate G:C→A:T and A:T→G:C transition, respectively. The results of Swenberg *et al* indicate that O^4-ethyl-thymidine is of equal or even higher significance to ethylating agents (144).

The mutagenicity of these lesions is modulated by a variety of factors, including DNA-repair. O^6-alkylguanine is repaired by O^6-methylquanine-DNA methyltransferase and the original guanine in DNA is restored by dealkylation (9). The methyltransferase accepts the alkyl group in one of its cysteine residues that results in its inactivation. A new protein must be generated prior to removal of another O^6-

alkyl adduct. The rate of resynthesis of this protein may therefore be a critical factor in the ability of the tissue to repair promutagenic damage prior to cell replication.

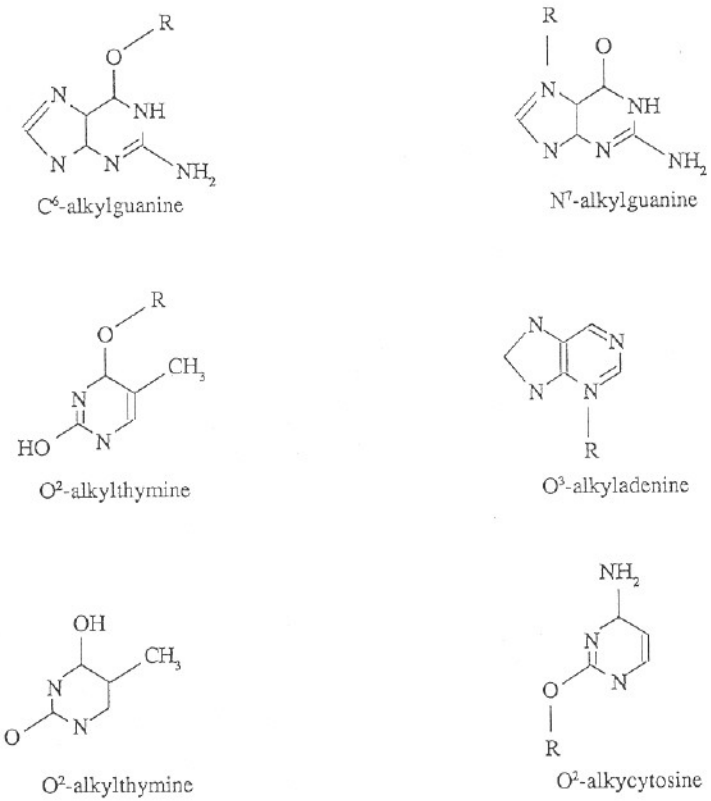


Fig 5 Example of DNA-adducts (structure)

The alkyltransferase pathway exhibits a much greater affinity toward short than long chain alkyl adducts (methyl > ethyl > butyl), and show only minor activity against O^4-alkyl-thymidines. Methyl DNA-adducts are removed 100 times more rapidly than n-propyl DNA adducts, and consequently the mutagenic adducts formed by the larger alkylating agent are likely to show a greater dependency upon repair by the excision repair pathway. Differential accumulation of damage in some tissues may reflect discriminatory alkylation repair which may contribute to the tissue-specificity exhibited by N-nitrosamine carcinogenesis.

The presence of O^6-methylguanine in human tissue has been reported previously in esophageal DNA of patients from Linxian county with cancer of the esophagus (153).

Higher concentrations of O^6-methylguanine were found in the Chinese cancer patients than in control population. Linxian county has a higher rate of esophageal and gastric cancer than other areas of China (Linxian is an agricultural area surrounded by the Tai Hang mountains) (161). A number of studies have found a higher level of environmental exposure to nitrosamines and their precursors in this region of China compared to other regions of the county (88). These data suggest an association between environmental nitrosamine exposure, formation of O^6-methylguanine and development of cancer. A second report shows the presence of O^6-methyl-guanine in DNA from human placenta (45).

Various antibodies are now available against many DNA adducts, and these have been used in sensitive immunoassays to detect these adducts at low levels in isolated DNA i.e. $1/10^7 - 10^8$ adduct/normal nucleoside (158,159).

6. ACUTE TOXICITY

The potency of N-nitroso compounds in causing acute toxicity (tissue injury and death) varies. Generally, these chemicals have a relatively low toxicity. However, many are acutely toxic, often leading to extensive liver damage. LD_{50} values in rats range from 18 mg/kg for N-nitrosomethylbenzylamine to more than 7.5 g/kg for N-nitrosomethyl-2-hydroxyethylamine (table 21). Acute toxicity seems to decrease with increasing chain length of the dialkylnitrosamines. Some cyclic nitrosamines are also acutely toxic.

Table 21 Acute toxicity of some N-nitroso compounds in rat (mg/kg)

N-nitroso compound	LD_{50}	Ref.
Nitrosamines		
Dimethylamine	27-41	63
Diethylamine	216	63
Di-n-propylamine	>400	113
Di-n-butylamine	1200	26
Di-n-amylamine	1750	27
Methyl-n-butylamine	130	63
Methyl-t-butylamine	700	63
Ethyl-n-butylamine	380	24
Ethyl-t-butylamine	1600	24
Ethyl-2-hydroxyethylamine	>7500	24
Di-2-hydroxyethylamine	>5000	127
Methylphenylamine	200	63
Methylbenzylamine	18	24
Nitrosamides		
Methylurea	180	24

7. GENOTOXICITY

Many N-nitroso compounds are potent mutagens (54). Mutagenesis by nitrosamines depends on their metabolism. In vitro bacterial system requires therefore a source of microsomes and NADPH. Nearly all of the carcinogenic nitrosamines have been shown to be mutagens. However, a

small group of noncarcinogenic nitrosamines are mutagens (54). Studies have shown that often very small changes in the chemical structure lead to major changes in the biological effects of nitrosamines. The higher homologue di-n-alkyl-nitrosamines tend to induce mutagenesis at lower doses than the lower homologues (54).

Higher homologue N-nitrosomethylamines are particularly potent over a large dose range probably because they are rapidly metabolised to methyldiazonium ion. Higher homologue cyclic nitrosamines are quite potent and tend to be more potent than lower homologues. The mutagenicity of most nitrosamines drops off dramatically when the number of carbons in the compounds exceeds 12-14. Furthermore, hydroxyl- and keto-groups generally reduce mutagenic activity.

8. CARCINOGENICITY

N-nitroso compounds are highly potent carcinogens. They are carcinogenic because they form metabolites which alkylate DNA (see chapter 5). Table 22 lists some of the N-nitrosamines and some sites where they induce tumors.

Since the first report in 1956 of the carcinogenic action of NDMA more than 300 nitroso-compounds have been tested for carcinogenic activity, and about 90% of them have been active (115).

Some N-nitroso compounds have been shown to be carcinogens in all 40 animal species tested, for instance NDEA. Other N-nitroso compounds have been tested in several species, usually with positive effects in all of them. There is strain specificity in the target organs for tumor development by a nitrosamine (86). As seen in table 22 the organs in which tumors araise can be quite different from one species to another. It is well known that the organotropic effects of N-nitroso compounds are sometimes dependent on the routes and

sites of administration.

The chemical structure influences both the carcinogenic potency and organotropy (table 23). This specificity was clearly demonstrated in early studies by Druckrey et al, involving 65 compounds (25).

Several dose-response studies have been published including low to very low exposure (table 24) (20,114,116,117).

Table 22 Carcinogenicity and target organs of N-nitroso compounds in different species

Target Organ	Chemical
Liver	
rat	NDMA,NDEA,NDPA,NDBA,NPYR,NPIP,NDELA,HNEU
mouse	NDMA,NDEA,NDBA,NPIP,MNNG
hamster	NDMA,NDEA,NPYR,HNEU
guinea pig	NDMA,NDEA,NDBA
Lung	
rat	NDPA,NDBA,NPIP,HNEU
mouse	NDMA,NDEA,NPYR,NPIP,MNNG,NNK,NNN
hamster	NDEA,NPYR,NDELA,NDBA,NNK
Esophagus	
rat	NDEA,NDPA,NPIP,NMBA,NNN,NAB
mouse	NDEA,NDBA,NMBA
Stomach	
rat	MNU,ENU,HNEU,MNNG
mouse	NDEA,NDBA,NPIP,NMBA,MNNG
hamster	HNEU,MNNG
Kidney	
rat	NDMA,NDEA
mouse	NDMA
Intestine	
rat	MNNG,HNEU,ENU
mouse	MNNG
hamster	MNU
Haematopoietic system	
rat	ENU,HNEU
Nasal cavity	
rat	NDELA,NNK,NNN
hamster	NDELA,NDPA,NNK,NNN
Nervous system	
rat	MNU,ENU,HNEU

Table 23 Effects of structure on carcinogenic potency and organotropy in rat

Class of Compounds	Potency	Example of organotropy: compound	organ
Symmetric dialkylamines	inverse related to chain length	NDMA	liver
		NDEA	liver, esophagus
		NDBA	liver, bladder
		NDCA	inactive
Asymmetric dialkyl-nitrosamines		Derivatives of NMA:	
		n-ethyl	liver
		n-propyl	esophagus
		n-pentyl	esophagus
		n-hexyl	esophagus
		n-octyl	bladder
Cyclic nitrosamines	related to ring size	N-nitrosoazetidine(weak)	
		- pyrrolidine (weak)	
		- pyrroline (weak)	
		N-nitrosopipridine	lung, esophagus
		N-nitrosomorpholine	
		N-nitrosoderivatives of hexa- and heptamethyl-eneimines	

Table 24 Dose-response studies. Lowest effective doses of N-nitrosamines (mg/kg).[1]

N-Nitroso-	Rat	Ref.	Mouse	Ref.
-dimethylamine	0.033	110	0.01	2
-diethylamine	0.033	110		
-piperidine	0.35	20		
-pyrrolidine	5	117		
-diethanolamine	15	116		

[1] Adapted from ref. 115.

Both nitrosamides and nitrosamines appear to induce neoplasms transplacentally. N-nitroso compounds induces tumors in the progeny of a variety of species (F1 generation) including rats, mice, hamsters and rabbits. Ivankovic and Druckrey reported transplacental carcinogenic effects of ENU after only one administration to the pregnant rat and also at the lowest dose (5 mg/kg) (75). The sensitivity of the fetus was about 10-30 times larger than the adult animals. The susceptibility to induction of tumors was greatest during late gestation and during early postnatal period. ENU also causes tumors transplacentally in monkeys. However, in monkeys, the period of greatest susceptibility is early gestation (121). Experimental reports also suggest an increased cancer risk in descendants (F2 generation) of MNU treated pregnant rats indicating that the tumors were the consequence of a heritable dominant condition (148). NDELA is a transplacental carcinogen in hamsters, rats and mice. Studies also suggest that tobacco smoke can act as transplacental carcinogen (1). Nicolov and Chernozemsky observed an increased frequency of benign and malignant neoplasms in offspring in Syrian golden hamsters injected i.p. with cigarette smoke condensate during pregnancy (99). Studies in humans demonstrate that the fetus of smoking parents is also exposed to compounds of cigarette smoke (78,92).

9. N-NITROSO COMPOUNDS AND HUMAN CANCER

9.1 Oral and respiratory cancer

There is a convincing epidemiological evidence associating N-nitroso compounds with human oral cancer. There is also an association between betel quid chewing, snuffdipping and buccal cancer. Furthermore, it seems likely that the NNK and NNN in tobacco products cause oral and respiratory cancer (8). Experimentally these compounds cause cancer in trachea, lung, and nasal cavity.

9.2 Esophagus

N-nitroso compounds may be involved in human esophageal cancer. High intake of N-nitroso compounds have been found in the area of very high esophageal cancer. Lin-xian food contains higher levels of N-nitrosamines than that from Fanxian (a low-incidence area for esophageal cancer)(135). However, the link between esophageal cancer and nitrosamine exposure is still very tennous.

9.3 Bladder

Epidemiological studies have indicated that bacterial infection of the bladder may be a risk factor for bladder cancer (72,79). For instance the high levels of N-nitroso compounds in the urine of Egyptian patients with schistosomiasis may relate to their incidence of bladder cancer (31). Ohshima et al have demonstrated that the concentration of N-nitroso compounds is elevated in the urine of patients with bacterial infections in the urinary tract (104). Studies show that several of the strains of bacteria involved, can mediate nitrosation reactions between nitrate and secondary amines under physiological pH.

9.4 Stomach

The incidence of gastric cancer varies from country to country (49). However, it is at the present time, decreasing throughout the world. There is a possible involvement of N-nitroso compounds in the etiology of this cancer (15). An excess in stomach cancer mortality has been reported for rubber workers (94). Intake of certain ethnic food may be important. Furthermore, gastritis may increase endogenous nitrate synthesis. For instance, chronic atrophic gastritis increases the pH and facilitates bacterial growth (18,19). However, there is no clear relationship existing between the total intake of nitrate and gastric cancer. Experimentally, the administration of nitrate and amines in the diet induces cancer in animals. The process of endogenous nitrosation in the stomach is complex. The process is influenced by many factors such as the nature of the nitrosable compounds, pH of the stomach, occurence of bacteria, and inhibitors and catalysts. Vitamins C and E have been shown to inhibit N-nitroso product formation by reducing nitrous acid. Wild et al have found detectable levels of O^6-methylguanine in stomach mucosa obtained surgically from patients in Lin-Xian (158).

10. TERATOGENICITY

N-nitroso compounds can also be potent teratogens. MNU given to rats on the 13th or 14th day of gestation results in fetal death, resorption and deformities. When ENU is given to rats before the 12th day of pregnancy, the compound is not carcinogenic (see chapter 8), but is a powerful teratogen (147).

11. RELATION BETWEEN EXPOSURE, EFFECT AND RESPONSE

There exists a great deal of uncertainty in predicting the potential response of exposure to chemicals at concentrations below those producing tumors in experimental animals. This is

particularly true when the response to the chemical in question is oncogenesis.

Acute toxicity of NDMA has been reported in exposed workers, necrosis and cirrhosis being observed in the liver (7,47,48). The lethal dose could not be determined exactly. In one of the cases, the authors assume that the patient received less than 1.5 g totally (48).

Cohen et al reported data indicating that the cytostatic N-nitrosourea derivatives, N,N-(bis-2-chloroethyl)-N-nitrosourea and N-cyclohexyl-N-2-chloroethyl-N-nitrosourea induced brain tumours 4 to 5 years after treatment (17).

It is well established that many N-nitroso compounds are potent carcinogens in animals inducing tumors at very low doses. There is no reason to believe that man should not be susceptible. Autrup and Stoner reported the ability of human tissue to metabolize N-nitroso compounds (6). Druckrey et al reported that no "treshold" could be detected in a dose-response study using NDEA. The lowest effective daily dose was 0.075 mg/kg. In table 24 some dose-response experiments are listed.

N-nitroso compounds have been detected in food, water, alcoholic beverages, tobacco, agricultural chemicals, urban air and certain occupational settings. Relatively high levels of preformed N-nitroso compounds have been found in industries such as leather tanning, rubber production and processing, manufacture and use of machining and grinding fluids, and in chemical industries. In some industrial settings the exposure of workers to NDMA and NMOR may exeed the exposure of N-nitroso compounds in contaminated food by a factor of 5000 (34,124). The highest levels of N-nitroso compound has been found in the rubber industry. Assuming $12m^3$ inhaled air per workshift and maximal observed exposure levels, the exposure level of NDELA may exeed 50 µg/day/person in the metal working industry, in leather tanning

industry, rubber and tyre industry and rocket fuel industry. Furthermore, in the rubber and tyre industry exposure levels >50 µg/day of NDEA, NMOR and NMPhA have been found. Exposure levels of >5 µg/day/person has been detected with NDBA (rubber and tyre industry, foundries, warehouse and sales-rooms), NDEA (foundries) and NMOR (warehouse and sales-room). Exposure levels of <5 µg nitrosamine/day/person has been detected of NDMA in dye manufactures, detergent and surfactant industry and fish-preserving industry (151). Metalworking industry, leather tanneries, and rubber and tyre industry have working environment with the highest nitrosamine exposure, i.e. NDELA and NMOR in the metalworking industry, NDMA in leather tanneries, and NDMA, NDEA, NDBA, NMOR and NMPhA in rubber and tyre industry (table 25).

Table 25 Occupational exposure to N-nitroso compounds

Industry	Major nitrosamines	Estimated exposure level (µg/workshift)	Ref.
Leather tanneries	NDMA	>50	124
Metal working industries	NDELA	>50	123
	NMOR		87
Rubber and tyre industry	NDMA	>50	123
	NDEA	>50	34
	NDBA	>5	136
	NMOR	>50	100
	NMPhA	>50	100
Chemical industries			
Rocket fuel	NDMA	>50	40
Dye manufacture	NDMA	<5	123
	NDEA	<5	123
Surfactant industry	NDMA	<5	123
Foundries	NDMA	>5	123
	NDEA	>5	137
Fish processing	NDMA	<5	123

The difference between the lowest doses in animals inducing tumours and human exposure levels is very small. Exposure to N-nitroso compounds should therefore be reduced. Ways of achieving this may be by not using tobacco products, changes in the industrial processes, regulatory and hygienic measures in occupational settings and by decreasing the levels of nitrosamines precursors in the environment (nitrate, nitrite, nitrogen oxides, easily nitrosatable amines).

12. NEEDS FOR FURTHER RESEARCH

A continued investigation of N-nitroso compounds is clearly wanted, both epidemiological and experimental animal studies. Cancer sites of primary interest are the stomach, esophagus, oral cavity, bladder and lung.

Further development of the assay on non-volatile N-nitroso compounds are needed. Today there is no reliable information on exposure to the non-volatile N-nitroso compounds because of analytical problems.

Further studies are needed on the combined effects of one or more carcinogen occurring together.

Sensitive biochemical monitoring methods to assess human exposure to N-nitroso compounds are needed. Urinary excretion products, DNA adducts and haemoglobin adducts are a possible direction.

Further studies should be made on the influence of N-nitroso compounds on structure and function of DNA.

13. SUMMARY

A. Haugen: N-nitroso compounds and cancer. Nordic Expert Group for Documentation of Occupational Exposure Limits. A review of current literature on N-nitroso compounds and cancer.

N-nitroso compounds are mutagenic, teratogenic, toxic as well as carcinogenic. The N-nitroso compounds are very versatile and potent chemical carcinogens. They induce tumors in a wide range of species and organs. Humans are exposed to N-nitroso compounds from endogenous formation and from environmental and occupational sources. Workers may be exposed to relatively high concentration of N-nitroso compounds in certain industries, such as metalworking industry, leather tanneries, and rubber and tyre industry.

Norwegian version is available, 162 references.

Key words: N-nitroso compounds, cancer, toxicology, genotoxicity, exposure, mutagenicity, carcinogenicity.

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Appendix I.

No occupational exposure limits have been set for nitrosamines in the Nordic countries, Holland, FRG, or the US. However, some of the countries have listed one or more as carcinogen or suspected human carcinogen.

Denmark (1988) ref.1	N-Nitrosodibutylamine N-Nitrosodiethanolamine N-Nitrosodiethylamine N-Nitrosodimethylamine N-Nitrosodipropylamine N-Nitrosoethylmethylamine N-Nitroso-N-ethylurea N-Nitroso-N-methylethylcarbamate N-Nitroso-N-methylurethane N-Nitroso-N-methylurea N-Nitrosomethylvinylamine N-Nitrosomorpholine N-Nitrosonornicotine N-Nitrosopiperidine N-Nitrosopyrrolidine N-Nitrososarcosine NNK	(Carcinogens. Handling only after special permission)
FRG (1988) ref.2	N-Nitrosodi-n-butylamine N-Nitrosodiethanolamine N-Nitrosodiethylamine N-Nitrosodimethylamine N-Nitrosodi-i-propylamine N-Nitroso-n-propylamine N-Nitrosoethylphenylamine N-Nitrosomethylethylamine N-Nitrosomethylphenylamine N-Nitrosomorpholine N-Nitrosopiperidine N-Nitrosopyrrolidine	(Carcinogens. Special precautions obligatory).
Sweden (1989) ref.3	N-nitrosomethylurea	(Carcinogen. Handling prohibited)
	N-nitrosodimethylamine	(Carcinogen. Can only be used after special permission)
USA (1989) ACGIH, ref.4	N-nitrosodimethylamine	(Suspected human carcinogen)
USA (1989) OSHA, ref.5	N-nitrosodimethylamine	(Carcinogen. Handling strictly regulated)

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Appendix II

Table 7 Non-volatile nitrosamines in meat products (µg/kg).

	NSAR	NPRO	NHPRO	NTCA	NOCA	Ref.
Smoked and unsmoked meat	<410	20-360	10-560	30-1620		149,150
Icelandic smoked mutton					40-70	149,150
uncooked cured meat				8-1400		109
fried bacon			>13700			133

Table 8 Volatile nitrosamines in meat products (µg/kg).

	NDMA	NPYR	NPIP	NHPYR	NTHZ	ref.
Various meat products	<5(30%)[1] >5(2.1%)	<5(6.8%) >5(6.8%)	<6.4(4%)			29
Fried bacon, ham and Bologna sausage				<7		76
Fried bacon					<5	53
Cured meat products				1.6-31.9 (16%)		108

[1] reported in 30% of the samples analysed

Table 9 Nitrosamines in fish and fish products (µg/kg).

	NTHZ	NPYR	NTCA	NPRO	NDMA	Ref.
Fish and fish products					0.1-1.0	156
						61
Japanese salt-dried fish					3.0-34	91
salted fish (coal gas broiled)					300	81
Raw squid					15-84	81
Raw squid (gas oven heated)		2.4-13			24-310	81
Japanese squid (boiled dried)		6		93.6	274	102
Salted chinese fish					6-20	46
Smoked oyster	109		167			67
Canadian fish			67			35

Table 10 Volatile nitrosamines in dairy products (µg/kg).

	NDMA	NPYR	NPIP	Ref.
cheese	0.5-0.9(14%)[1]			
	1-6 (10%)			29
dried milk	<0.2			
	1.7			30
	0.1-3.7	0-0.8	0-0.5	85
				62

[1] Reported in 14 % of the samples analysed

Table 11 Volatile N-nitroso compounds in vegetables (µg/kg).

	NDMA	NPYR	NEMA	NDEA	Ref.
Japanese fermented vegetables	<5	<5			80
Pickled vegetables in Wuwei county, China	2.16		3.10	0.69	162
Clear-soup pickled vegetables	11.44		5.12	4.9	162
Brine fermented green mustard leaves, Chinese cabbage, radish roots and stem	0.6-13.0	2.4-96			112
Dried and pickled vegetables from Kashmir	0.25-1.85				134
Dried spices	2.75				134
Dried chilli powder	15.5	6.1			152

Table 12 Alchoholic beverages (µg/l).

	NDMA	Ref.
Beer	2	29
	5.9	126
	0.44	118
Scotch whisky	0.2-2	50
Apple brandy	0.6	155

ORGANIC ACID ANHYDRIDES

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CONTENTS

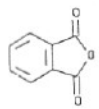
BACKGROUND

Organic acid anhydrides are widely used in the chemical industry, especially in the manufacture of plastics. The workers are exposed to acid anhydrides either during their production or when they are used in the production of polymers. This document deals mainly with phthalic anhydride and trimellitic anhydride. Other acid anhydrides possessing comparable toxicological characteristics are mentioned whenever there are data available.

1. PHYSICAL AND CHEMICAL DATA

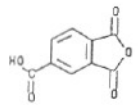
The data in this chapter are taken from references 2, 32, 55, 84 and 88.

PHTHALIC ANHYDRIDE (PA)

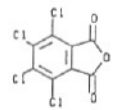
CAS	85-44-9
Systematic name	1,2-benzenedicarboxylic acid anhydride
Synonyms	1,3-isobenzofurandione 1,3-dioxophthalan 1,3-phthalandione phthalic acid anhydride
Molecular formula	$C_8H_4O_3$
Structural formula	
Form of existence	white chrystalline needles
Molecular weight	148.12
Melting point	131.16°C
Boiling point	284-295°C
Density	1.527 (4°C)

Vap.press./20°C	0.3×10^{-4} kPa
Air odour threshold	0.32mg/m^3
Conversion factors	1 ppm= 6.046 mg/m^3 1 mg/m^3= 0.165 ppm

TRIMELLITIC ANHYDRIDE (TMA)

CAS	552-30-7
Systematic name	1,2,4-benzenetricarboxylic acid-1,2-anhydride
Synonyms	trimellic acid anhydride
Molecular formula	$C_9H_4O_5$
Structural formula	
Form of existence	white flakes
Molecular weight	192.12
Melting point	161-163.5°C
Boiling point	240-245°C
Sublimation point	390°C
Vap.press./20°C	$< 0.9 \times 10^{-9}$ kPa
Conversion factors	1 ppm = 7.842 mg/m^3 1 mg/m^3 = 0.128 ppm

TETRACHLOROPHTHALIC ANHYDRIDE (TCPA)

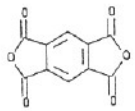
CAS	117-08-8
Molecular formula	$C_8Cl_4O_3$
Structural formula	
Form of existence	white, odorless,free-flowing nonhygroscopic powder
Molecular weight	285.88

Melting point	254-255°C
Boiling point	371°C
Conversion factors	1 ppm = 11.669 mg/m^3
	1 mg/m^3 = 0.086 ppm

MALEIC ANHYDRIDE (MA)

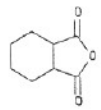
CAS	108-31-6
Systematic name	cis-butanedioic anhydride
Synonyms	2,5-furandione
	toxilic anhydride
	maleic acid anhydride
Molecular formula	$C_4H_2O_3$
Structural formula	
Form of existence	colorless needles
Molecular weight	98.06
Melting point	53°C
Boiling point	202°C (sublimates)
Density	1.48
Vap.press./20°C	0.7 x 10^{-5} kPa
Air odour threshold	1.23mg/m^3
Conversion factors	1 ppm = 4.002 mg/m^3
	1 mg/m^3 = 0.250 ppm

PYROMELLITIC DIANHYDRIDE (PMDA)

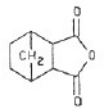
CAS	89-32-7
Molecular formula	$C_6H_2(C_2O_3)_2$
Structural formula	
Form of existence	white powder
Molecular weight	218.12
Melting point	286°C

Boiling point	397-400°C
Conversion factors	1 ppm= 8.903 mg/m^3
	1 mg/m^3= 0.112 ppm

HEXAHYDROPHTHALIC ANHYDRIDE (HHPA)

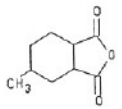
Systematic name	1,2-cyclohexandicarboxylic anhydride
Molecular formula	$C_8H_{10}O_3$
Structural formula	
Form of existence	clear, colorless, viscous liquid
Molecular weight	154.17
Boiling point	158°C
Density	1.19 (40°C)
Conversion factors	1 ppm = 6.293 mg/m^3
	1 mg/m^3 = 0.159 ppm

HIMIC ANHYDRIDE (HA)

Systematic name	3,6-endomethylene 4 tetrahydrophthalic anhydride
Molecular formula	$C_9H_{10}O_3$
Structural formula	

METHYLHEXAHYDROPHTHALIC ANHYDRIDE (MHHPA)

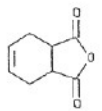
Systematic name	4-methylcyclohexyl-1,6-dicarboxylic anhydride
Molecular formula	$C_9H_{12}O_3$

Structural formula 

Form of existence oily liquid

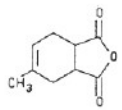
Molecular weight 168.19
Boiling point 120°C
Conversion factors 1 ppm = 6.865 mg/m^3
1 mg/m^3 = 0.146 ppm

TETRAHYDROPHTHALIC ANHYDRIDE (THPA)
CAS 85-43-8
Molecular formula $C_8H_7O_3$

Structural formula 

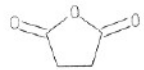
Molecular weight 152.16

METHYLTETRAHYDROPHTHALIC ANHYDRIDE (MTHPA)
CAS 26590-20-5
Molecular formula $C_9H_{10}O_3$

Structural formula 

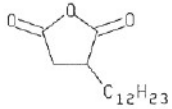
Molecular weight 166.19
Conversion factors 1 ppm = 6.783 mg/m^3
1 mg/m^3 = 0.147 ppm

SUCCINIC ANHYDRIDE
CAS 108-30-5
Molecular formula $C_4H_4O_3$

Structural formula 

Form of existence colorless needles
Molecular weight 100.08
Melting point 119.0°
Boiling point 261°
Density 1.104
Conversion factors 1 ppm= 4.881 mg/m^3
1 mg/m^3= 0.205 ppm

DODECENYLSUCCINIC ANHYDRIDE
CAS 25377-73-5
Molecular formula $C_{16}H_{26}O_3$

Structural formula 

Form of existence light-yellow, clear viscous oil
Molecular weight 266.42
Boiling point 180-182°
Density 1.002
Conversion factors 1 ppm= 10.874 mg/m^3
1 mg/m^3= 0.092 ppm

2. USE AND OCCURRENCE

2.1 Use

Phthalic anhydride (PA) is used in the production of alkyd resins, plasticizers, hardeners of resins, polyesters, and in the synthesis of phenolphthalein and other phthaleins, many other dyes, chlorinated products, pharmaceutical intermediates, insecticides, diethylphthalate, dimethylphthalate and laboratory reagents.

PA was first synthetized by oxidation of naphthalene. After 1960 the process has been changed to oxidation of o-xylene. The purified product contains 99.9% PA, 0.01% phthalic acid, 0.01% MA and 0.02% benzoic acid.

Trimellitic anhydride (TMA) is used as a plasticizer for polyvinylchloride, alkyl coating resins, high-temperature plastics, in wire insulation, gaskets, and automobile upholstery.

Tetrachlorophthalic anhydride (TCPA) is used as an intermediate in dyes, pharmaceuticals, plasticizers and other organic materials and as flame retardant in polyester resins, hardener for epoxy resins.

Maleic anhydride (MA) is used in the production of polyester resins, alkyl coating resins, fumaric and tartaric acid manufacture, pesticides, preservatives for oils and fats, permanent-press resins (textiles) and in Dies-Alder reactions.

Pyromellitic dianhydride is used as a curing agent for epoxy resins for high temperature laminates, molds and coatings, as a cross-linking agent for epoxy plasticizers in vinyls, alkyl resins and as an intermediate for pyromellic acid.

Hexahydrophthalic anhydride (HHPA)is used as an intermediate for alkyds, plasticizers, insect repellents and rust inhibitors and as a hardener in epoxy resins.

Himic anhydride (HA) is used in the production of fire retardants.

Methylhexahydrophthalic anhydride (MHHPA) is used as a hardener in epoxy resins.

Tetrahydrophthalic anhydride (THPA) is used in the production of unsaturated polyester resins and alkyl resins with increased resistance to water and solvents.

Methyltetrahydrophthalic anhydride (MTHPA) is used as a hardener in epoxy resins.

Succinic anhydride is used in the production of synthetic adhesive resins, alkyl resins, elastomers, lubricants, pharmaceuticals, photographic chemicals, plastics and resins, synthetic fibres and textiles.

Dodekenylsuccinic anhydride is used in alkyl, epoxy and other resins, anticorrosive agents, plasticizers, and wetting agents for bituminous compounds.

(32, 84, 88, 96)

2.2 Occupational exposure

The concentrations of PA, TMA and HHPA measured in the workplace air are presented in Tables 1-3. The concentration of HA in the flame retardant production has been measured to be less than 0.5mg/m^3 (detection limit)(82). In the epoxy resin coating environment, the MTHHPA concentration has been measured to be 0.1mg/m^3 as a time-weighed average (60).

Table 1. Phthalic anhydride (PA)

Type of exposure	PA concentr.(mg/m³). mean	range	Ref.
Alkyl resin polymerization			66
open system			
- PA powder throwing	153.3	not given	
- floor cleaning	475.9	"	
- air escaping from scaling tank		452.5-495.6	
- " " " storage tank	177.3	"	
closed system		not det.-<6.8	
Di(2-ethylhexyl)phthalate (DEHP)production			48
- DEPH production	0.038	0.006-0.102	
- PA "	0.053	0.004-0.187	
- PA and DEPH maintenance	0.024	0.011-0.026	
- batch ester plant production	0.011	0.005-0.021	
- " " " maintenance	0.033	0.021-0.044	
- tank farm	0.079	0.017-0.203	
Polyester resin production			101
Plant A			
- loading of reactors	4.9	0.3-15	
- handling empty bags	13	6.8-23	
- cleaning	<0.3	<0.1-0.6	
Plant B			
- loading of reactors	2.8	2.3-3.2	
- assist.loading,incl.handling empty bags	6.1	1.5-12	
- cleaning	0.3	<0.1-0.6	
- general work	0.15	<0.1-0.4	
- in canteen (area sampling)	<0.1	<0.1-0.2	
PA and unsaturated polyester resin production			72
- flaking	1.49	1.26-1.62	
- sacking	0.52	0.32-0.72	
- flaking (process.difficulties)	2.95	2.34-3.56	
- sacking " "	1.18	0.98-1.38	
Working of polyvinyl plastics			72
- calendering (165°C)	0.0003	not given	
- extrusion (180°C)	0.0003	"	
- welding (220°C)	0.005	"	
Polyester resin production			59
Plant A: loading of reactors	6.1	1.8-14.9	
other work	<0.1		
Plant B: loading of reactors	6.8	1.5-17.4	
other work	<0.1		

Table 2. Trimellitic anhydride (TMA)

Type of exposure	TMA concentr.(mg/m³) mean	range	Ref.
Paint and varnish company		0.1-7.5	49
TMA production			109
- condensing molten TMA (fume)	1.7	not given	
- pouring waste products (fume)	1.8	"	
- bagging area (dust)	4.7	"	
- warehouse area (dust)	3.3	"	
Powder paint exposure			46
- powder room operator	1.7		
- " " "	3.6		
Mixing epoxy resin			12
1974-78 operator	2.1		
assist.operator	0.82		
packager	0.007		
1979 operator	0.006		
assist.operator	0.002		
packager	0.08		
1984 operator	<0.04		
assist.operator	<0.04		
packager	0.32		

Table 3. Hexahydrophthalic anhydride (HHPA)

Type of exposure	HHPA concentr.(mg/m³) mean	range	Ref.
Manufact. buchings for electr. transformers.			56
-mixing towers, mold pouring	3.74	1.26-8.18	
-mold stripping,finishing	1.89	0.63-3.15	
-other locat.,HHPA area	1.89	1.26-2.52	
-locat.adjacent to HHPA area	1.89	not given	

2.3 Methods to analyze acid anhydrides from workplaces

Phthalic anhydride (PA)

NIOSH presents the following method to measure PA (S179): A known volume of air is drawn through a cellulose membrane filter to trap the organic aerosol present. The filter is treated with ammonia; the anhydride is hydrolyzed to the acid, which is analyzed in a high performance liquid chromatograph (HPLC) equipped with a 254 nm UV detector. The detection range is 1-36 mg/m^3 (62).

Geyer and Saunders sampled PA with a glass fiber filter. Desorption and hydrolysis was carried out using sodium hydroxide. Phthalic acid was measured with reverse phase HPLC with a UV-detector. The minimum quantifiable amount corresponded to 0.5 mg/m^3 using a 100 l air sample (28).

Pfäffli sampled PA from air with Tenax polymer tubes and analyzed PA with a gas-chromatographic method utilizing a ^{63}Ni electron-capture detector. The limit of detection was 0.0004 mg/m^3 (0.00007 ppm) with an air sample of 12 l (72).

Trimellitic anhydride (TMA)

Palassis and coworkers have provided a method to measure TMA (64, 67). They sampled air on a PVC-copolymer membrane filter. After methanol and boron trifluoride treatment, the adduct was analyzed in a gas chromatograph equipped with a flame ionization detector. The detection limit was 0.002 mg.

Geyer and coworkers collected samples on glass fiber filters and converted TMA with 0.05 M sodium hydroxide solution to the corresponding acid. The analysis was carried out with HPLC. The minimum quantifiable amount was 0.001 mg on a filter sample or 0.000004 mg injected into the HPLC (26).

Maleic anhydride (MA)

NIOSH presents a method to measure MA, No: P&CAM 302. A known volume of air is drawn through a midget bubbler containing 15 ml of distilled water. Maleic acid is analyzed by HPLC with a UV-detector. The limit of detection is estimated to be 0.015 mg MA per sample. (63)

Geyer and Saunders used a similar method with 0.1% phosphoric acid in distilled water as absorbing solution and mobile phase. The minimum quantifiable amount was 0.1 mg/m^3 of MA using a 100 l air sample (27).

3. TOXICOKINETICS

3.1 Uptake

It may be assumed that the main route of exposure is via the respiratory system either in powder or gas form.

There are no data on absorption via the gastrointestinal system or skin.

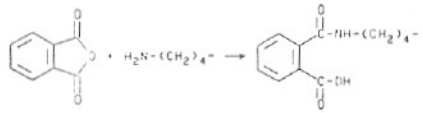
After exposing rats for 2-11 weeks to 34.3, 68.6 or 137 mg/m^3 of MHHPA vapour, dose-dependent blood anhydride concentrations of MHHPA were measured. The maximum concentrations were found after two weeks. (87).

3.2 Distribution

There are no data available.

3.3 Biotransformation

PA and MA react with water to form a corresponding acid (51). Other acid anhydrides probably react similarly. Exposure-dependent concentrations of phthalic acid have been measured in human urine, and of methylhexahydrophthalic acid in the urine of rats (73, 87). MHHPA was, however, found in the circulation of rats immediately after the exposure, which may indicate that the rate of spontaneous hydrolysis to the acid is not very rapid (87).
Conjugation with human serum albumin (HSA) takes place in the hapten formation of acid anhydrides (58, 109). Hapten means small chemical, which is not immunogenic on its own, but when linked to a carrier protein becomes immunogenic (81).



PA HSA PA-HSA

In vitro at 37°C TMA was conjugated with HSA rapidly; the reaction was essentially completed in one minute (109).

3.4 Elimination

Acid anhydrides are excreted into urine as the corresponding acids (dicarboxylic acids). Pfäffli followed the excretion of phthalic acid in workers exposed to PA, by taking urine samples pre-shift, on-shift, post-shift and also in the evening and on the following morning. At low atmospheric exposure to phthalic anhydride (0.15 mg/m^3, range 0.03-0.33 mg/m^3) the pre-shift concentrations were on the same

level as the phthalic acid concentrations found in the urine samples of occupationally unexposed people (0.34 mmol/mmol creatinine, range 0.02-0.89 mmol/mmol creatinine).Higher concentrations (1.63 mg/m^3 ± 0.13SD) of PA resulted in a body load of phthalic acid. The pre-shift phthalic acid excretion was 1.02 ± 0.25 mmol/mmol creatinine. When the exposure was high, 10.5 mg/m^3, the pre-shift urinary concentration was 4.8 mmol/mmol creatinine, about 14 times that of the value of workers with low exposure. No conjugation of phthalic acid to glucuronide was observed (73).

The half-life of phthalic anhydride/phthalic acid was shown to be about 14 h (73). The assumed half-life of the dicarboxylic acids in urine after low exposure to MHHPA was about 7 h and to HHPA and THPA about 14 h. After 4 h exposure to 0.116 mg/m^3 of MHHPA in the air, an input-output equilibrium for the anhydride and the urinary acid was developed (74).

3.5 Biologic monitoring

Measurement of the dicarboxylic acids in urine can be used to reveal exposure to organic acid anhydrides. Pfäffli has reported a method to determine the dicarboxylic acids of phthalic anhydride, hexahydrophthalic anhydride, methylhexahydrophthalic anhydride and tetrahydrophthalic anhydride. The corresponding detection limits were 15-2-3-4 mg/m^3 respectively (74).

When concentrations of PA are 2mg/m^3 or lower, the peak concentration in the urine can be measured after the shift. With higher concentrations, sampling times immediately after the shift and 6 h later are recommended. A pre-shift sample gives information of the possible body burden (73).

4. GENERAL TOXICOLOGY

4.1. Acute toxicity in animals

Table 4 presents compiled data on the acute toxicities of organic acid anhydrides in different species of mammals.

Table 4. Acute effects of acid anhydrides on different animal species.

Species	Route of administration	Response	Dosage	Ref.
			mg/kg	
Phthalic anhydride				
rats	oral	LD_{50}	4020	61
guinea pigs	oral	LD_{LO}	100	61
mice	oral	LD_{50}	1500	61
mice	ip	LD_{50}	75.5	16
mice	ip	LD_{01}	54.8	16
cats	oral	LD_{50}	800	61
Trimellitic anhydride				
mice	oral	LD_{50}	1250	4
rats	oral	LD_{50}	5600	61
rats	oral	LD_{50}	1900	4
Maleic anhydride				
rats	ip	LD_{50}	97	61
guinea pigs	oral	LD_{50}	390	61
mice	oral	LD_{50}	465	61
rabbits	oral	LD_{50}	875	61
rabbits	skin	LD_{50}	2620	102
Tetrahydrophthalic anhydride				
mice	ip	LD_{LO}	500	61
Methyltetrahydrophthalic anhydride				
rats	oral	LD_{50}	2140	93
rabbits	skin	LD_{50}	1410	93

Abbreviations:
LD_{50}: a calculated dose, which is expected to cause the death of 50% of an experimental animal population.
LD_{LO}: the lowest dose reported to have caused death.
LD_{01}: a dose which is expected to cause the death of 1% of an experimental animal population.

4.2 Mechamisms of toxicity

Acid anhydrides are mucous membrane and skin irritants (3, 4, 11, 22, 51, 59, 92). They can cause allergic skin disorders and induce conjunctivitis, rhinitis and asthma by immunological or nonimmunological mechanisms (35, 59, 89, 109). TMA has caused severe "pulmonary disease-anaemia" syndrome, which is also based on an immunological mechanism (109). The effect of oral TCPA (25, 100, 250 or 500 mg/kg in corn oil) on hepatic microsomal metabolism has been studied in rats and mice. Oral TCPA was a weak wide-spectrum inducer of microsomal enzymes in the rat; a similar effect was not observed in the mouse (80).

5. EFFECTS ON ORGAN SYSTEMS

5.1 Skin and mucous membranes

5.1.1 Irritation

PA and MA, when cold, are not very harmful to dry skin, but hot compounds may cause severe chemical burns. On sweating skin, both are hydrated to acids and can cause caustic dermatitis and burns. A part of the irritant effect of PA may be caused by MA, which PA contains as an impurity (51, 54). PA solution (50%) in oil did not irritate rabbit ears after 20 hours of exposure (25). PA (0.5 g/patch) did not cause skin irritation to rabbits by the semiocclusive or occlusive method in one or four hours. The results were assessed at 1, 24, 48 and 72 h, or 7 days later (77).

TMA (50%, 2h) caused dermatitis to mice and rats after a single or repeated application to the skin. The symptoms, however, were slight and reversible (4)

Irritation of mucous membranes has been usual in workers exposed to PA (3, 11, 59).

One drop of PA (5%) in polyethyleneglycol 400 was slightly irritating to rabbit eyes, while a 0.5% solution was not irritating (25).

MA and TMA have been extremely irritating to eyes in animal experiments. There was cloudiness of the cornea and hyperemia of the conjunctiva a few minutes after application of 1% MA to the eyes of rabbits; on the next morning the eyes were normal. A 5% solution of MA induced more intense irritation, which lasted one week. A minute amount of MA powder caused long-lasting damage with vascularization of the cornea of the rabbit (103). Application of 50 mg of TMA powder to rabbit eyes produced reversible hyperemia of conjunctiva, lacrimation and blepharospasms. (4).

In a six-month inhalation study on MA, rats, hamsters and monkeys were exposed to concentrations of 0, 1.1, 3.3 and 9.8 mg/m^3, 6 hours a day on 5 days a week. Ocular irritative signs were present at all exposure levels. (92).

In an experiment with rabbits, the irritant effect of PA on the skin and eyes correlated with each other; PA was found to be a mild skin irritant, but a moderate eye irritant (22).

5.1.2 Sensitization of the skin

There are few reports of delayed type contact allergy to acid anhydrides. Nevertheless, PA is claimed to be a primary sensitizer (18). The potency of PA to induce allergic contact dermatitis has been investigated with the Buehler Test (closed Patch test in guinea pigs) and

the mouse ear swelling test (MEST). With both tests PA was classified as a moderate sensitizer (21). The guinea pig maximization test has not been carried out with acid anhydrides.

In a material over a ten-year period from the Department of Occupational Dermatology, of the Finnish Institute of Occupational Health, comprising 542 cases of allergic occupational contact dermatitis, no cases due to acid anhydrides were found (38). There is only one case report of allergic contact dermatitis due to acid anhydrides. Dodekenylsuccinic anhydride sensitized a laboratorty technician who prepared tissues for electron microscopy. The delayed type allergic reaction was verified with positive patch tests with 0.5 and 1% dodekenylsuccinic anhydride in acetone. Tests to fifteen controls were negative. The patient did not react to epoxy resin, accelerator or another hardener (30).

Both Menschick (54) and Baader (3) reported urticarial reactions in workers in PA production. There is, however, only one case report of immediate type dermatitis due to acid anhydrides. Methylhexahydrophthalic anhydride (MHHPA) induced contact urticaria to a worker from a factory where electronic components were filled with MHHPA-cured epoxy resin. The sensitization was confirmed with the open test, which gave a positive reaction with the filling mixture and the hardener, tested separately. Open tests with all the other components were negative, as well as the epicutaneous tests with all components. Open tests with the hardener in 12 control persons were negative (38).

5.2 Respiratory system

5.2.1 Irritation

Nasal and bronchial irritation, presenting as sneezing, coughing and dyspnea, is a common feature in exposure to acid anhydrides (3, 54, 59).This has been reported also in animal experiments. In a six-month inhalation study with MA, rats, hamsters and monkeys were exposed to 0, 1.1, 3.3 and 9.8 mg/m^3. Nasal irritation was seen at every exposure level. Examination of nasal tissue revealed irritation (hyperplasia, metaplasia) or inflammatory changes (92).

The human nasal irritation threshold of PA is 30 mg/m^3 and of MA 5.48 mg/m^3 (83).

5.2.2 Asthma and rhinitis

Occupational asthma caused by an organic acid anhydride was first reported by Kern in 1939 (39). During the last ten years a growing number of asthma cases due to different acid anhydrides has been reported. The symptoms have been those of typical occupational asthma; after a latent period the worker gets asthmatic symptoms when exposed to acid anhydrides at the workplace. Rhinitis often precedes the asthmatic symptoms. The diagnosis has been based on the history, and the cause-effect relationship has been proven with different types of challenge tests and/or an immunological test. Both tests were not always used (98). The asthmatic reactions in the challenge tests have been immediate, late or dual ones. The reports are presented in Table 5 according to the exposure; the anhydrides in order after the first published case. The immunological background will be described later in section 6.2.

Table 5. Reports on asthma due to acid anhydrides

Exposure	Industry/occupation	Concentration mean (range) mg/m^3		Ref.
PA	paint factory/chemist			39
	varnish production			24
	chemical foreman		*	50
	tire and rubber manufacture			14
	paint factory/resin plant operat.			17
	meat wrapper, price label fume			47
	plastic grinder			97
	resin production	(2.8-13)		101
	PA production			57
	resin production	6.6 (1.5-17.4)	*	59
TMA	epoxy powder painting			17
	production of TMA	(1.7-4.7)	*	109
	mixing TMA powder to epoxy res.		*	86
	epoxy powder painting	(1.7-3.6)	*	46
TCPA	epoxy resin production			89
	epoxy powder coating		*	35
MA	epoxy resin insulation			29
			*	94
PMDA	manufacture and use of epoxy adhesive			53
HHPA	epoxy resin molding		*	56
HA	manufacture of synthetic flame retardants	< 0.5	*	82
MHHPA	filling electric components	(0.04-2.75)	*	40
MTHPA	epoxy resin coating	0.1	*	60

* Specific IgE against the acid anhydride has been found in these studies.

There are only few follow-up studies on asthma due to acid anhydrides. When six TCPA-sensitized workers without any further exposure were studied for four years, all were reported to experience continuing asthmatic symptoms pointing to a poor prognosis (100).

5.2.3 Clinical syndromes caused by TMA

TMA is a unique chemical known to cause four different clinical syndromes reported first in 1977.

1 Immediate type TMA-asthma begins after a latent period. The worker gets asthmatic symptoms and often rhinitis immediately after the exposure. Specific IgE is found. (70, 109).

2 Late onset respiratory system syndrome begins after a latent period. The worker gets cough, wheezing and dyspnea, starting 4-8 hours after the exposure. The respiratory symptoms are accompanied by malaise, chills, fever, myalgia and arthralgias. No IgE antibodies are found, but IgG and IgA antibodies (70, 109).

3 The pulmonary disease-anaemia syndrome is severe and even fatal. It has occurred only in workers exposed to fume containing TMA produced by spraying hot pipes with a resin containing TMA. The symptoms are hemoptysis, dyspnea, pulmonary infiltrates, restrictive ventilatory function, hypoxemia and anaemia. IgG antibodies have been found to both human serum conjugate and erythrocyte conjugate. Open lung biopsy has showed intact alveolar septae, but extensive intra-alveolar haemorrhage with granular pneumocyte hyperplasia (1, 34, 70, 79, 104, 109). This syndrome has not been reported to be caused by any other chemical.

4 The direct irritant syndrome to "high" levels of fume. The symptoms begin after the first exposure; rhinorrhea, cough and dyspnea, lasting up to eight hours. The reaction is not immunologically mediated (109).

5.3 Liver

There are no reports dealing with liver toxicity by acid anhydrides.

5.4 Kidney

When rats, hamsters and monkeys were exposed to MA vapor 1.1-9.8 mg/m^3 for six months, no exposure-related effects were seen (92).

5.5 Gastrointestinal system

Ulceration of the gastric mucous membrane has been found in rats with heavy intragastric exposure (more than 640 mg/kg) to PA (20).

5.6 Heart and blood vessels

When rats were exposed intragastrically to PA (20 to 4800 mg/kg daily over 9 weeks), congested capillaries were found in the heart muscle. Otherwise the heart muscle was well perserved. (20).

5.7 Blood and blood-forming organs

When guinea pigs were exposed to PA dust (8.5 mg/m^3) 3 h per day for four days, and after ten days a new exposure period up to 8 months, no changes in erythrocyte or thrombocyte count or in white blood cells were found. (20).

In a six-month animal experiment with 1.1-9.8 mg/m^3 of MA vapour, an increased amount of hemosiderin was seen in the spleens of high-dose (9.8 mg/m^3) female rats. However, there was no histological (bone marrow) or clinical evidence of red blood cell destruction (92).

5.8 Nervous system

When rats were exposed to 34.3, 68.6 or 137.3 mg/m^3 of MHHPA vapour (6 h daily, 5 days weekly during 2-11 weeks), their cerebral and cerebellar acetylcholinesterase activity was below the control range at 137.3 mg/m^3 after two weeks ($p < 0.01$). After 11 weeks the activities were on the same level. Creatin kinase activity increased in the cerebellar tissue after 11 weeks of exposure ($p < 0.01$) (87).

6. ALLERGY AND IMMUNOTOXICITY

6.1 Skin

Allergic reactions of the skin are rare. However, both delayed and immediate type skin reactions have occasionally been observed (see 5.1.2).

6.2 Respiratory system

6.2.1 Immunological findings in asthma and rhinitis due to acid anhydrides

The proof of IgE mediation in an immediate type asthma or rhinitis due to acid anhydrides is very convincing. When Kern in 1939 reported the first case of asthma and rhinitis due to PA, he already had evidence of the immunological background. The scratch test with PA in crystalline form and diluted 1:1000 in alcohol gave positive reactions. The tests in control patients were negative. The passive transfer test was also positive (39). Maccia and his colleagues were the first to find specific IgE in the serum of a patient with asthma due to PA. The patient got an immediate asthmatic reaction in the provocation test with PA dust (50).

Specific IgE has now been found to phthalic anhydride, trimellitic anhydride, maleic anhydride, tetrachlorophthalic anhydride, hexahydrophthalic anhydride, himic anhydride, methylhexahydrophthalic anhydride and methyltetrahydrophthalic anhydride (Table 6). Rast inhibition studies have supported the specificity. However, cross-reactivity between different acid anhydrides has been observed (94).

The half-life of specific IgE after leaving the work with exposure has been one year (58).

Immediate type skin tests with acid anhydride-HSA conjugate have correlated well with the finding of specific IgE in immediate type asthma (35, 65, 69, 110).

An IgE-mediated reaction does not, however, explain all of the cases with occupational asthma or rhinitis due to acid anhydrides. Other immunological or nonimmunological mechanisms are involved (59, 65, 89, 98.

The location and specificity of the IgE antibody for the epitopes present on the acid anhydride(hapten)-protein complex have been studied. Epitope is the contact area of the antigen with an antibody (81).

It has been postulated that the reaction of acid anhydride with albumin altered the albumin to form new antigenic determinants, or that hapten is altered at the antibody-combining site (5, 69, 107, 110). The formation of new antigenic determinants on the albumin site explains the cross-reactivity in the RAST tests.

There is evidence that, for the patients sensitized to TCPA and TMA, the antibody combines with the anhydride and the adjacent portion of the HSA molecule, whereas in the patients sensitized to PA, the antibody is

specific to the hapten (94). TMA is claimed to form unique antigenic determinants that do not bind significantly with antibodies formed by sensitization to PA, HHPA and HA. This may explain why significant cross-reactivity with it has not been found in the inhibition studies (6, 94, 110).

Specific IgG antibodies have been studied especially in connection with sensitization to TMA. Specific IgG to TMA-HSA antibodies have been correlated with late onset occupational asthma due to TMA. They have also been found in the Pulmonary Disease Anemia Syndrome due to TMA, as well as IgG antibodies to erythrocyte conjugate (68, 70, 71, 86, 95).

Specific IgG_4 antibodies have been associated with work-related symptoms in PA-exposed workers (59, 65). Nielsen and coworkers have studied specific IgE, IgG, IgG_4 and IgM antibodies to PA in workers from alkyd and/or unsaturated polyester resin production. The level of exposure was 6.6 (1.5 to 17.4) mg/m^3 (TWA). Of the heavily exposed workers 69% had rhinitis or conjunctivitis. Five workers (14%) had PA-induced asthma. There was a significant ($p = 0.01$) difference only of specific IgG against PA between the heavy and low exposure groups. Only one worker with asthma had an increased specific IgE level. The subjects with asthma had significantly higher values for specific IgG than the asymptomatic subjects. Specific IgG_4 was found in four persons; three had asthma and one rhinitis. The writers conclude that specific IgG is an index of PA exposure, and that specific IgG_4 may be a pathogenetic factor in asthma (59).

Specific IgA antibody activity to TMA has been found in most TMA-exposed workers with or without symptoms (86).

The total antibody activity to TMA-HSA has correlated well with specific antibody activity. When 20 workers exposed to TMA were evaluated, IgG and total antibody activity against TMA discriminated symptomatic workers from the asymptomatic ones (86).

Venables and her coworkers have studied the effect of smoking and atopy on antibody E production in 300 TCPA-exposed workers. Atopy was defined as at least one positive skin prick test to common allergens. Twenty-four of the workers had specific IgE to TCPA. Twenty of the 24 (83.3%) were current smokers compared with 133 (48.2%) of 276 without antibody ($P<0.01$). Atopy was also more common in those with specific IgE, but not significantly so. Smoking and atopy interacted, the prevalence of antibody being 16.1% in atopic smokers, 11.7% in nonatopic smokers, 8.3% in atopic nonsmokers ($p > 0.025$). The writers conclude that smoking may predispose to and interact with atopy in the production of specific IgE antibody to TCPA (99).

There are some findings of mediator release in acid anhydride sensitivity. When the basofil leukocytes were challenged in vitro with PA or TCPA-HSA conjugates, the release of histamine, a mediator of allergic reaction, was found. The in vitro histamine assay was claimed to be useful in the identification of subjects with allergic responses to anhydrides even without evidence of IgE-mediated reaction (19).

Animal experiments

When monkeys were exposed parenterally with PA-monkey serum albumin (MSA), PA dissolved in ethanol-saline, MSA or ethanol-saline alone, sensitization was observed only with PA-MSA. The presence of new antigenic determinants formed by PA on protein carriers was essential for parenteral sensitization (8).

Dykewicz and coworkers (15) sensitized two rhesus monkies intrabronchially with the sera of a worker with TMA asthma and high titers of IgE, IgG and IgA to trimellityl(TM)-HSA. The monkies were challenged with TM-HSA aerosol and bronchospasm was demonstrated. After one week the challenge was negative. The passive cutaneous anaphylaxis was also demonstrated with the Prausnitz-Küstner test.

When guinea pigs were exposed to TMA fume 0, 1, 15 and 100 mg/m^3 (3h/day/5 days), IgG_1-anti TMA antibodies were detected in the majority of the animals exposed to the two higher concentrations. Specific IgE was found in very few animals. Challenge with TMA-guinea pig serum albumin, however, induced no increase in the respiratory rate in any of the animals (9).
When guinea pigs were sentitized by a single intradermal injection of 0.1 ml of TMA in corn oil into the nape of the neck, IgG_1 anti-TMA antibodies could be found in the sera of all 40 TMA-injected animals and IgE anti-TMA-antibodies in the sera of 12-40 (with different methods) of 40 animals. The sera of 24 corn oil injected control animals were negative in all assays. All animals were challenged on day 24 with free TMA (5.8-52.3 mg/m^3, with an atomizer) or with TMA-GPSA (0.9-2.8 mg/m^3, with a nebulizer). Moderate to severe pulmonary responses were seen. TMA caused more severe reactions than TMA-GPSA; this was supposed to be due to the different generation system. There was no clear relationship between IgG_1 or IgE antibody titre and the severity of the pulmonary reaction. Responses were often seen in the absence of detectable IgE-anti-TMA antibody.

In these two studies different responses were found according to the way of exposure, which was supposed to reflect a difference in the dose of chemical available

for the stimulation of an immune response. The writers suggest avoidance of dermal exposure following spills or splashes at the workplace, in addition to the respiratory protection (10).

6.2.2 Immunological findings in TMA-induced "Pulmonary Disease-Anaemia syndrome" (PD-A)

The first case of this new disease was reported by Rice et al in 1977. Herbert and Orford found seven more in 1979 and Ahmad et al another two cases in 1979. All had been exposed to fumes from TMA-cured epoxy resin applied on hot pipes. The symptoms were cough, haemoptysis, dyspnea, pulmonary infiltrates, restrictive respiratory defect, hypoxaemia and anaemia. The symptoms varied from mild to very severe. A possible fatal case was identified (1, 34, 68, 79). Extensive studies to find out the mechanism of this disease have been carried out.

Patterson et al did not find any IgE antibody activity against TM-HSA in workers with PD-A. IgG activity against TM-HSA did not differ from the level in other workers exposed to TMA fumes under similar working conditions. IgG, IgA and IgM antibodies against TM-human erythrocytes were found (68). Antibodies to TM-human erythrocytes were also found in workers with asthma due to TMA but not in non-exposed persons (95).

Animal experiments

In an animal experiment dogs and rabbits were immunized intrabronchially with TMA 0.1, 1.0 and 10.0 mg/kg (powdered TMA was suspended in 1 ml of physiologic saline and instilled through a catheter inserted through a cuffed endotracheal tube). Predominantly IgG and lower levels of IgM and IgA antibodies against

trimellityl-erythrocytes were detected in both species. Lymphocyte reactivity was present in all dogs in the lymphocyte transformation test. Some of the dogs exposed to low and moderate doses of TMA died, whereas none of the dogs exposed to the high dose died. No correlation of deaths could be made with serum antibody levels or lymphocyte activity. Autopsy findings showed haemorrhagic pneumonitis possibly analogous to the pulmonary lesion seen in some workers exposed to TMA fumes. None of the rabbits died (85).

In an inhalation experiment rats were exposed to 0, 0.01, 0.03, 0.1 and 0.3 mg/m^3 of TMA dust. Haemorrhagic lung foci were found related to the exposure concentration from 30 to 300 ug/m^3. Serum antibody binding of trimellityl-rat serum albumin correlated with the exposure concentration, haemorrhagic lung foci and lung weight. The lung lesions healed 12 days after exposure but returned soon after a repeated exposure (108).

Leach et al exposed rats to TMA dust (particle size 1.11 um, concentrations 0, 0.01, 0.03, 0.1 and 0.3 mg/m^3) for 6 h per day, on 5 or 10 exposures. After 5 exposures there were no macroscopic or histological effects or antibody response. After 10 exposures lung weights had increased in proportion to the concentration. Numerous areas of focal haemorrhage were seen over all lobes, but not on the conducting airways. The number was related to the TMA concentration. Histologic examination indicated extensive cellular infiltration, primarily macrophages, alveolar haemorrhage, pneumonitis and haemoglobin crystals. These effects increased in proportion to the concentration. Lung and mediastinal lymph node nonspecific IgG and complement also increased in proportion to the concentration. There were no findings in other organs except the lungs (45)

Chandler et al exposed rats to TMA powder 0.1 mg/m^3 for six h per day, five days a week for two weeks. Haemorrhagic foci were observed on the surface of the lungs at autopsy. The authors found higher total antibody concentrations in the fluid of bronchoalveolar lavage (BAL) than in the serum. There were IgG, IgA and IgM antibodies to TM-rat serum albumin. Inhibition studies showed that the early antibody response in the rat was directed toward new antigenic determinants common to TMA-modified albumins (13).

When Leach et al exposed rats to 0.095 mg/m^3 of TMA (6 h per day, 5 days per week for two weeks) with or without cyclophosphamide as an immunosuppressant, the cyclophosphamide-treated rats showed no lung lesions and no antibody reaction. The spleen cells of cyclophosphamide-treated rats showed little blastogenic response. Thus the elimination of T- and B-cell function could prevent the TMA lesions (44).

Zeiss and coworkers exposed rats to TMA (TMA powder 0.1 mg/m^3 6 h/day for 2, 6 or 10 days) and found that the immune response to inhaled TMA occurs parallel with the development of lung lesions. The antibody levels in BAL and serum were highly correlated with the lung injury (106).

When rats were exposed to TMA via inhalation 0.1 mg/m^3 6h per day for 2,6 or 10 days, IgG antibody levels to TM-conjugated haemoglobin rose througout the exposure both in the serum and in the BAL fluid. The response correlated also with IgG response to TM-rat serum albumin (76).

7. MUTAGENICITY AND GENOTOXICITY

No mutagenic activity has been seen with PA or TCPA in Salmonella typhimurium in the Ames test (105). No effect with PA or TCPA was seen when chromosome aberrations in cells derived from Chinese hamster ovary cells or rat liver cells were studied in vitro (23, 75).

Furthermore, when Chinese hamster ovary cells were tested for the induction of sister chromatid exchanges, tests with PA and TCPA were also negative (23).

8. REPRODUCTION TOXICITY AND TERATOGENICITY

Fabro et al studied the teratogenicity of PA and succinic anhydride in mice with daily ip injections at doses of 0.2 to 0.6 mmol/kg/day on pregnancy days 8-10. PA was found teratogenic only at the level of maternal toxicity. SA induced a significant incidence of malformations at doses within the maternal lethality range (16).

No reproductive effects were observed for TMA in CD-1-mice when TMA 550 mg/kg was given orally throughout days 7 to 14 of gestation (33).

No treatment-related effects on the fetal development were seen in rats treated orally with 140 mg/kg/day MA in corn oil from days 6-15 of gestation. Furthermore, no treatment-related effects on reproduction were observed with MA at doses up to 55 mg/kg/day over two generations (91).

PA caused malformations at a high frequency when 0.2, 0.1, 0.05 or 0.025mg of PA per egg was injected in the air chamber of the egg of three-day chicken embryos. A dose-response effect for early deaths was seen (43).

9. CARCINOGENICITY

Information on the carcinogenicity of acid anhydrides is very scarce.

When six rats where injected subcutaneously twice weekly with 2 mg/succinic anhydride/animal in 0.5 ml arachis oil for 65 weeks, subcutaneus sarcomas developed at the injection site in all of the three rats that survived 93-106 weeks. No tumours occurred in the 24 controls injected with arachis oil alone that survived 45-106 weeks.(36).

No evidence of carcinogenicity of phthalic anhydride was found in long-term feeding studies in rodents (31, 41, 42, 90).

In a case-referent study mortality from lung cancer was investigated in an acetylene and phthalic anhydride plant. After control for age and smoking, the lung cancer mortality odds ratio for the subjects exposed in the factory was 5.6. The corresponding odds ratio in other work environments of the region was 1.7. There was, however, exposure also to phthalates and soot (78).

Succinic anhydride is the only acid anhydride which has been evaluated by the International Agency for Research on Cancer (IARC): there are no adequate data for its human, and limited evidence for its animal carcinogenicity. It is not classifiable as carcinogenic to humans (37).

10. EXPOSURE, EFFECT AND RESPONSE

10.1 Short-term exposure

The general toxicity of phthalic anhydride and trimellitic anhydride has been low in animal studies (4.1.). The data on the effects of short-term exposure on eyes, skin and on the immunological response are presented in Table 6.

Table 6. Animal data from short-term exposure studies

Dosage	Exposure time	Effect/response (species)	Ref.
PHTHALIC ANHYDRIDE			
5%	eye application	reversibly irritating to eyes (rabbit)	25
0.5%	eye application	no irritation to eyes (rabbit)	25
500 mg/ patch	one or four h (semiocclusive or occlusive)	no skin irritation (rabbit)	77
TRIMELLITIC ANHYDRIDE			
50 mg	eye application	conjunctival hyperaemia, lacrimation (rabbits)	4
0.1ml of 30%TMA	one intradermal injection	IgG_1 and IgE anti-TMA antibodies. Severe reactions in bronchial challenge with TMA (guinea-pigs)	10

10.2 Long-term exposure

The data of the effects of PA and TMA on eyes and respiratory system reported from the workplaces is collected in Table 7. The results of animal studies of long-term exposure to PA, TMA and MA are presented in Table 8.

Table 7. Human data from long-term exposure studies (D = dust, F = fume)

Concentr.mg/m^3 mean	range	No. of exposed	Exposure time	Effect/response No. of cases (%)	Ref.
PHTHALIC ANHYDRIDE					
	2.8-13 (D)	118	1 mo-16 yrs	rhinitis 28 (24%) asthma 21 (18%)	101
6.6 (D)	1.5-17.4	35	0-43 yrs	conjunctivitis 16 (46%) rhinitis 14 (40%) asthma 4 (14%)	59
$\leq$ 0.2 (D)		25	0.3-40 yrs	conjunctivitis and rhinitis 13(52%) asthma 0	59
TRIMELLITIC ANHYDRIDE					
(F) and (D)	1.7-1.8 and 3.3-4.7	not giv. 16 with symptoms	1 mo-11 yrs	irritation 6 rhinitis and immediate asthma 4 immediate and late asthma 8.	109
	1.7-3.6 (F and D)	9	months to 10 yrs	irritation 4 asthma 3	46
	0.01-2.1 (D)	18	8.6 yrs	rhinitis 1 late asthma 3	7
<	0.001-0.1 (D)	11	2 yrs	none with symptoms and no antibody response	52

Table 8. Animal data from long-term inhalation exposure studies (D = dust, F = fume)

Concentration (mg/m^3)	Exposure time	Effect/response (species)	Ref.
PHTHALIC ANHYDRIDE			
8.5 (D)	3h/day/4 days, 10 days without exposure/ 8 months	hyperemia of mucous membranes of respir. tract (guinea pigs)	20
TRIMELLITIC ANHYDRIDE			
15-100 (F)	3 h/day/5 days	IgG_1anti-TM antibodies, (specific IgE in few animals (guinea pigs)	9
1 (F)	3 h/day/5 days	no antibody induction (guinea pigs)	9
0.03-0.3 (D)	6 h/day/5 or 10 days	hemorrhagic lung foci and TM-MSA antibodies after 10 days (rats)	108
0.1 (D)	6 h/day/5 d/ week/two weeks	hemorrhagic foci on lungs, IgG, IgA and IgM antibodies to TM-RSA in sera and BAL fluid (rats)	13
0.01 (D)	6 h/day/5 or 10 days	no effect on lungs (rats)	108
MALEIC ANHYDRIDE			
1.1-9.8	6h/day/5 days/week/ 6 months	nasal and ocular irritation, metaplasia of nasal mucosa (rats, hamsters and monkeys)	92

In animal studies, one single injection has induced an immunological response with positive bronchial provocation tests. When workers were exposed to a concentration less than 0.2 mg/m^3 of PA dust, no asthmatics were found. However, half of the workers had

conjunctivitis or rhinitis. When exposed to 0.001-0.1 mg/m^3 of TMA dust, none of the workers got symptoms or revealed an antibody response.

Succinic anhydride injected subcutaneously has induced subcutaneous sarcomas in rats (36). The International Agency for Research on Cancer (IARC) has stated that there is limited evidence for the animal carcinogenicity of succinic anhydride (37).

11. RESEARCH NEEDS

Very little is known of the toxicokinetics of PA in animals, and even less of other acid anhydrides.

Longitudinal epidemiologic studies with exposure measurements are needed to clarify the dose-effect relationship between exposure levels, symptoms and immunological response. The mechanism of the sensitization to acid anhydrides is not fully understood.

Long-term inhalation studies with organic acid anhydrides are needed to evaluate carcinogenicity.

12. DISCUSSION AND EVALUATION

The effects caused by acid anhydrides are induced by direct exposure of the skin or the mucous membranes or via the respiratory system. The main effects are irritation and sensitization. Despite the chemical differences, often minimal, every organic acid anhydride coming into use has seemed to be able to induce sensitization of the respiratory system. Information on the exposure levels of acid anhydrides in workplaces where workers have become sensitized is very limited. Most reports are clinical case reports without measurements of the actual concentrations.

PHTHALIC ANHYDRIDE

The critical effects of exposure to PA are respiratory symptoms, sensitization and irritation.

When 35 workers had been heavily exposed to PA (6.1-6.8 mg/m^3) for a mean 12 years on average, 16 (46%) had conjunctivitis, 14 (40%) rhinitis and five (14%) had asthma. 25 workers had had only slight exposure (0.1-0.2 mg/m^3 of PA). Of these 13 (52%) had conjunctivitis or rhinitis. No asthmatics were found. The workers in the former group had been exposed to high concentrations during loading of the reactors, which lasted about 30 min/day. During the rest of the day, the exposure was much lower. When a time-weighted average for a full day was calculated, it was approximately 0.4 mg/m^3, well below the Swedish occupational exposure limit value at the time. The symptoms were probably caused by the exposure peaks (59).

The levels at which PA sensitization has been induced in workers are quite low when compared to the occupational exposure limit in most countries. Despite the low levels, there are exposure peaks which probably induce the sensitization, as reported by Nielsen (59). However, the recent occupational exposure limit seems to be too high.

Symptoms of irritation of the mucous membranes among the workers have been usual even at low levels of exposure to PA, 0.1-0.2 mg/m^3; well below the recent exposure limit values (59).

TRIMELLITIC ANHYDRIDE

The critical effects of exposure to TMA are respiratory symptoms, irritation or sensitization. TMA probably

induces immunologically mediated immediate and late type asthma and rhinitis and the severe pulmonary disease-anaemia syndrome.

All nine workers from a barrel manufacturing plant were investigated because of their exposure to TMA. The personal breathing zone levels for TMA were 1.7 to 3.6 mg/m^3. One worker was asymptomatic. Four workers had TMA-induced irritant effects. Three had symptoms and IgG levels consistent with TMA late respiratory system syndrome. One of them had also specific IgE against TMA. One worker had bronchitis not related to TMA (46).

The exposure levels of TMA, which have induced occupational asthma, have been high when compared to the recent occupational exposure limit of 0.04 mg/m^3. There is no information on exposure levels when the pulmonary disease-anaemia syndrome due to TMA fume is induced. All of these cases were exposed to TMA fume induced by spraying hot pipes with a resin containing TMA. In animal studies, however, the corresponding syndrome has been induced by exposure to TMA dust; 0.03-0.3 mg/m^3 concentrations of TMA dust have induced haemorrhagic lung foci and antibody production, while 0.01 mg/m^3 did not (108).

In animal studies TMA in powder form has been more effective than the fume in causing an immunological response (9, 13, 108). Different work hygienic values for fume and powder should be discussed.
One intradermal injection has in animal studies caused a more potent immunoresponse than exposure by inhalation for several days. Thus, avoidance of skin contact with these reactive chemicals may be important (10).

MALEIC ANHYDRIDE

The critical effects of exposure to MA are irritation of the mucous membranes and respiratory symptoms (29, 92, 94, 103). It has induced nasal metaplasia in long-term inhalation exposure in animal studies (92). The information of respiratory sensitization due to MA is limited. Specific IgE has been found, however (29, 94).

SUCCINIC ANHYDRIDE

The data are insufficient to assess the critical effect of SA in man. However, when rats were subcutaneously injected with SA during 65 weeks, they developed subcutaneous sarcomas (36). Therefore there is some evidence of the carcinogenic potency of SA in experimental animals.

13. SUMMARY

A critical survey of the literature relevant to the discussion of occupational exposure limits for organic acid anhydrides is presented. Organic acid anhydrides are used in manufacturing processes in the plastic industry. The exposure can occur via skin, mucous membranes and the respiratory system.

PHTHALIC ANHYDRIDE

Critical effects are respiratory sensitization and irritation of the mucous membranes.

TRIMELLTIC ANHYDRIDE

Critical effects are respiratory sensitization and irritation of the mucous membranes.

MALEIC ANHYDRIRE

Critical effects are irritation of the mucous membranes and respiratory sensitization.

SUCCINIC ANHYDRIDE

The data are insufficient to assess the critical effects of SA in man. There is some evidence that succinic anhydride is carcinogenic to animals.

When occupational exposure limits for organic acid anhydrides are discussed, their irritating effect and the sensitization of the respiratory system should be the main points considered.

Number of references: 110

Key words: organic acid anhydrides, phthalic anhydride, trimellitic anhydride, irritation, allergy, rhinitis, conjunctivitis, asthma, pulmonary-disease anaemia syndrome, occupational disease, occupational exposure limit.

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APPENDIX I. Occupational exposure limits to phthalic anhydride, trimellitic anhydride and maleic anhydride.

Occupational exposure limits to phthalic anhydride

Country		mg/m^3	ppm	Year	Ref.
FRG	MAC	5		1988	6
Denmark		5		1988	3
Finland		2		1987	8
	STEL	3			
France		6		1987	11
Great Britain		6	1	1987	7
	STEL	24	4		
Iceland		5		1978	9
Netherlands		6	1	1989	5
Norway		5		1988	2
Soviet Union	MAC	1		1978	10
Sweden		6	1	1989	4
	STEL	12	2		
USA	(ACGIH)	6	1	1988-89	1
	(OSHA)	6	1	1989	12

Occupational exposure limits to trimellitic anhydride

Country		mg/m^3	ppm	Year	Ref.
FRG	MAC	0.04	0.005	1988	6
Denmark		0.1		1988	3
Finland					
France		0.04	0.005	1987	11
Great Britain		0.04		1987	7
Iceland					
Netherlands		0.04	0.005	1989	5

Norway	0.04	0.005	1989	2
Sweden CLV	0.04	0.005	1989	4
USA (ACGIH)	0.04	0.005	1988-89	1
(OSHA)	0.04	0.005	1989	12

Occupational exposure limits to maleic acid anhydride

Country	mg/m^3	ppm	Year	Ref.
FRG MAC	0.8	0.2	1988	6
Denmark	0.8	0.2	1988	3
Finland	1	0.25	1987	8
STEL	3	0.75		
France	1		1987	11
Great Britain	1	0.25	1987	7
Iceland	0.8	0.2	1978	9
Netherlands	1	0.25	1989	5
Norway	0.8	0.2	1989	2
Soviet Union	1		1978	10
Sweden	1.2	0.3	1989	4
STEL	2.5	0.6		
USA (ACGIH)	1	0.25	1988-89	1
(OSHA)	1	0.25	1989	12

Abbreviations:

MAC: maximum allowable concentration
STEL: short-term exposure limit
CLV: ceiling limit value

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STYRENE

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CONTENTS

INTRODUCTION

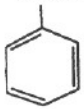
Styrene, also known as vinylbenzene, is a colorless, viscous liquid which has a characteristic pungent odor. Styrene is used in the production of polymers, copolymers, and together with unsaturated polyester resins, in the production of reinforced plastics. Some exposure occurs in styrene/polystyrene manufacturing plants and in operations involving the working and application of styrene-containing polymers (102). The highest levels of exposure are, however, found in the industries applying unsaturated polyesters with styrene in the reinforced plastics industry.

The exposure levels of the general population are usually considerably lower than occupational exposure levels (108). Industrial sources are the most likely cause of general population exposure. Other potential sources of exposure include motor vehicle exhausts, tobacco smoke, and other combustion/pyrolysis products.

The health effects of styrene have been extensively reviewed (102,108); the present document is an update of the styrene document from the Nordic Expert Group from 1979 (164).

1 PHYSICO-CHEMICAL DATA

CAS number:	100-42-5
Synonyms:	ethenylbenzene, vinylbenzene, phenylethylene, phenylethene, cinnamene, styrol
Physical state (25°C) and appearance:	clear, colorles, viscous liquid with a pungent odor

Molecular formula:	C_8H_8
Molecular structure:	$HC=CH_2$ 
Molecular weight:	104.15
Boiling point (101.3 kPa):	145.2°C
Freezing point:	-30.63°C
Flash-point:	31°C
Density (20°C):	0.906
Vapor pressure (25°C):	0.86 kPa
Refractive index:	nD^{20} 1.5463
Spectroscopy data:	X_{max} 246 nm (ϵ = 1514)
Odor threshold:	(water) 0.01 ml/l (air) 0.2 mg/m^3 (0.05 ppm)
Conversion factors for air concentrations (20°C, 101.3 kPa):	1 mg/m^3 = 0.235 ppm 1 ppm = 4.26 mg/m^3
Solubility:	320 mg/l in water; miscible with ethanol, diethylether, methanol, acetone, and carbon disulphide

2 USES AND OCCURRENCE

2.1 Usage

Styrene is polymerized as such to polystyrene or with other monomers, butadiene and acrylonitrile, to corresponding copolymers, or it is used as a reactive solvent for other polymers (21,23).

The main part of the styrene produced is used for styrene-containing thermoplastics; in the 80s the world capacity was about 6 x 10^6 tons. The largest outlet for polystyrene (about 40 %) is in packaging applications. Specific uses include bottle caps, small jars and other injection molded or blown containers, vacuum formed articles toughened with polystyrene as liners, and oriented polystyrene films.

Polystyrene and high-impact polystyrene (butadiene-styrene) are widely used in the manufacture of housewares, for example refrigerators, containers, toys, sports equipment, radio and electrical equipment, and bathroom fittings. Butadiene-styrene is also used in the production of water-latexes used as coatings for paper and carton. Styrene-butadiene-elastomers are used in the making of tires.

Polystyrene is used also in buildings and industrial constructions, as well as in vehicle construction where, especially, acrylonitrile-butadiene-styrene terpolymers have the greatest applicability. Expanded polystyrene that is not used for packaging is used for thermal insulation in refrigeration equipment and in buildings.

Styrene is also used as a reactive diluent for unsaturated polyester resins (40 % of styrene in the resin blend) which are further used in the production of reinforced plastic goods, e.g. boats, airplane and

car cabins and other parts for vehicles, containers, sheets, tubes, and also in paints and cements.

2.2 Occupational exposure

Occupational exposure to styrene may occur in the following situations: in the production of styrene, in the polymerization of styrene as such, or with other monomers, in the handling and transporting of liquid styrene or prepolymers, in the working of thermoplastics containing styrene, in the production of polyester resins containing styrene as a reactive solvent, as well as the preparation of reinforced plastic goods from polyesters, and in the production and usage of paints or cements prepared from polyester resins.

The commercial synthesis of styrene and polymerizing processes are generally performed in closed kettles, thereby limiting the exposure to the filling, opening and maintenance operations. With careful industrial hygienic management in normal working conditions, the time-weighted average (TWA) exposure may be minimized (below 20 mg/m^3; 5 ppm) in these situations.

Styrene polymerization is an exothermic reaction with a possible ultimate temperature of 300°C in the reactor. At such temperatures precautions must be taken to prevent the occurrence of uncontrolled polymerization reactions. Accidents can arise if condensed styrene vapor, which is free of inhibitor, polymerizes and forms popcorn polymer, leading to blockages in pipes and valves.

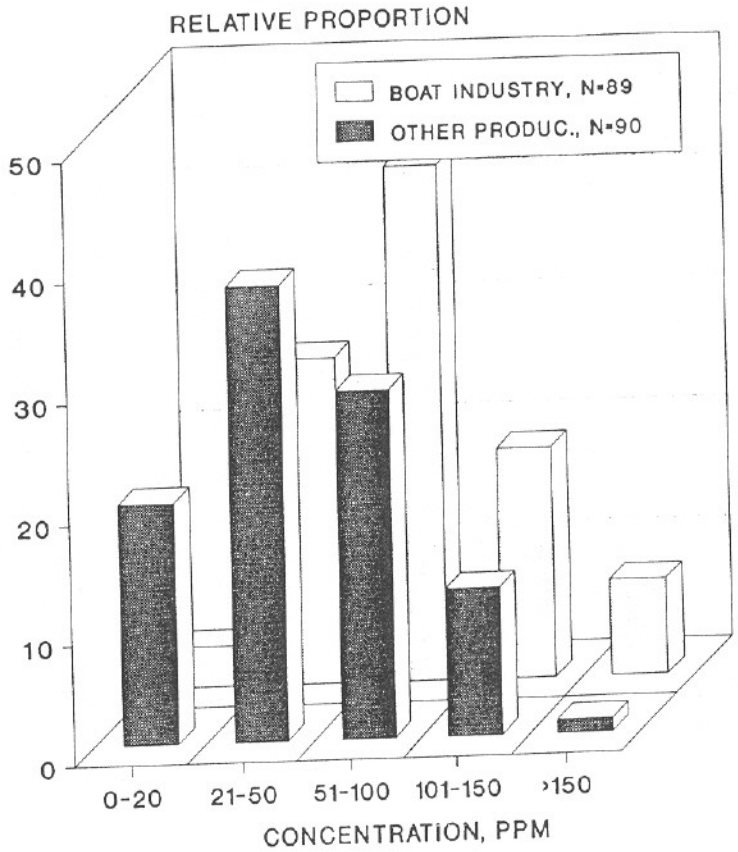
Styrene (including expanded polystyrene without fire retardant) is inflammable, thus also causing a fire hazard in work with open flames, electric heaters,

static electricity and other sources of ignition.

In work with thermoplastics containing styrene, the exposure is developed from the thermal degradation process of the heated plastic material. The random degradation of polystyrene macromolecules produces small amounts of styrene monomer. At working temperatures (about 200°C), however, the degradation is so slow, that the concentrations of exposure remain usually lower than one ppm (90).

The highest occupational styrene exposures are found in the production of glass-reinforced polyester goods (cf. Fig. 1). The commercial unsaturated polyester consists of linear glycol phthalate with styrene as a crosslinking agent. During fabrication, organic peroxides are added as initiators, and cobalt compounds and tertiary amines as regulators and stabilizers of the exothermic polymerizing reaction. Styrene evaporates into the work place air during application procedures.

In the 60s and early 70s, industrial hygienic measurements in the reinforced plastic industry were rarely done and/or published. In the measurements of the Institute of Occupational Health in Finland, the styrene concentrations in the personal samples of laminators (hand and spray-up applications) were in the range of 850-1280 mg/m^3 (200-300 ppm), whereas the average was about 425 mg/m^3 (100 ppm). The measurements were mainly performed during the worst exposure moments of the work processes. In the 80s the average concentrations in similar samples were slightly lower than in the previous decade (Fig. 2), but were still clearly higher (mean 300 mg/m^3; 70 ppm) than the occupational exposure limit (in Finland 8 h TWA 210 mg/m^3 (50 ppm) in 1982). In the boat industry, the 8 h TWA exposures varied between 50 and 100 ppm, while in other kinds of polyester plastics industries



N = number of samples

Figure 1. Workers' exposure (TWA, 8h) to styrene in glass reinforced polyester industry in Finland, years 1980 - 1986

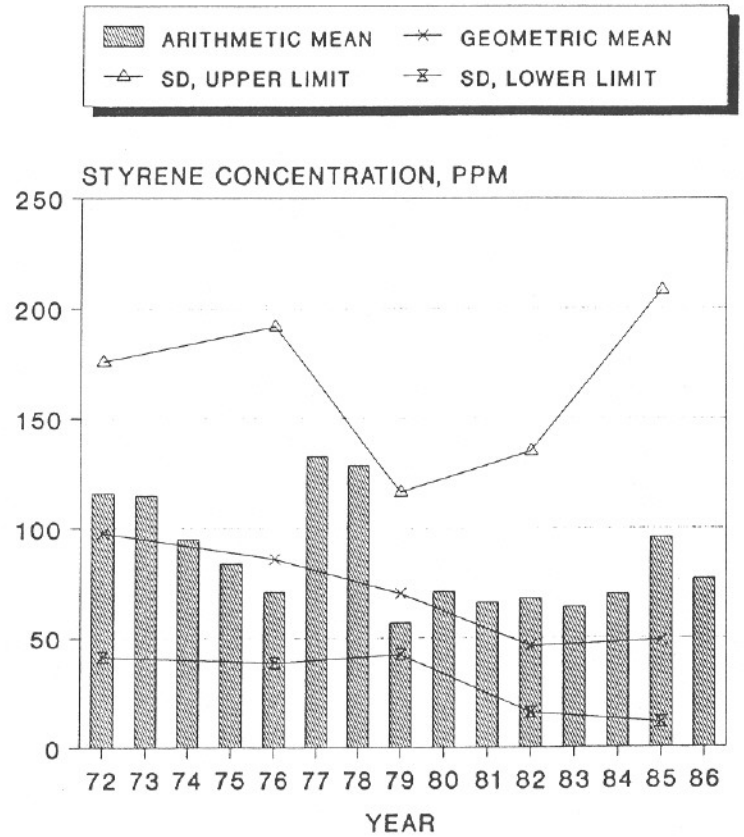


Figure 2. The annual averages of styrene exposure measured from personal samples of workers in reinforced plastic industry

more than 50 % of the measurements remained lower than 210 mg/m^3 (50 ppm). Through the years 1980 to 1986 the majority of the measured concentrations in the reinforced plastic industry were higher than the current Finnish hygienic reference value of 20 ppm (85 mg/m^3) (1987).

2.3 Measurement of styrene in the air

One approach to the sampling of the atmospheric concentrations of styrene is to collect direct grab samples with evacuated glass flasks or metal containers, or use bags made of polar plastic material or use sampling syringes (14,239). Collection can also be carried out by impingers containing liquids, and analyses performed either by gas chromatography or by spectrometry (122,250).

The above-mentioned methods are better suitable for ambient air measurements than for personal samplings, for which sampling tube collections operated by battery-driven pumps are suitable (25,55,114,161). The essential principle of the tube sampling is to collect the air impurity (styrene) on a solid material from a known volume of air pumped through the tube. Coconut charcoal, organic polymers or silica have been used as adsorption materials. After elution of the analyte from the solid adsorbent with solvents (e.g. carbon disulfide) the analysis can be performed by gas chromatography with a flame ionization detector.

Diffusive charcoal samplers are also available for personal samplings without active pumping ("passive samplers") (105). The sampling badges can be worn throughout the work day, for instance for measurement of 8 h exposure. The analyses can be performed in the laboratory in a similar way as in the case of

adsorption tubes.

Continuous monitoring of airborne styrene concentrations in the work place atmosphere can be performed with an infrared spectrometer equipped with a variable path length gas cell and a pump. The recommended wavelengths for quantitative measurements are 11.0 and 13.0 µm (225).

3 TOXICOKINETICS

3.1 Uptake

3.1.1 Uptake by inhalation

Pulmonary uptake is the main route of occupational exposure to styrene. Styrene uptake and absorption has been the subject of a number of human inhalation studies (10,63,64,67,68,220). In these studies the mean pulmonary uptakes of styrene ranged from 59-89 %.

The arterial levels of styrene in humans increase as a function of increased concentration in the air and the rate of ventilation (63,252). Blood concentrations of styrene were measurable shortly after the onset of exposure, indicating rapid absorption of styrene across the alveolar membrane into the blood (252).

Metabolites of styrene, styrene-7,8-oxide and styrene glycol can be measured in the blood of workers exposed to styrene in glass-fiber reinforced plastic factories (138). The mean exposure level was 99 mg/m^3, and the concentration of styrene-7,8-oxide measured in most of the blood samples was 0.02 µmol/l, which is at the detection limit.

The absorption of styrene by the lungs of rats is rapid (194,243,244). The blood levels of styrene were measurable within minutes following exposure, and increased during the 5-hour exposure to levels of ~1.5, 40, 150 and 200 µg/ml for exposure concentrations of 50, 500, 1200 and 3000 ppm, respectively (244). Equilibrium levels of styrene in the blood of 0.8, 1.5, 25 and 64 µg/ml corresponding to concentrations of 80, 200, 600 and 1200 ppm, respectively, were reached generally within 6 hours during a 24-hour exposure study (193,194). In mice, styrene oxide and styrene glycol have been measured in several tissues after intraperitoneal injections of radioactive styrene (136). The levels of styrene oxide were highest in subcutaneous fat, but were measurable in several tissues including liver, lung and kidneys. In general, the levels of styrene oxide in tissues were less than 10% of the levels of styrene glycol (136).

3.1.2 Uptake through the skin

The rate of absorption of styrene through the skin is very low, about 0.06 mg/cm^2/h when measured after dipping a hand into liquid styrene (16). The skin absorption of styrene in its vapor phase is negligible (195,240).

The dermal penetration rate of styrene in rats was measured <u>in vitro</u> by applying 1 ml of styrene to 2.55 cm^2 of excised rat abdominal skin in a diffusion cell (226). The penetration rate was estimated as 4.8 nmoles/min/cm^2 (0.3 mg/cm^2/h) after a 1.67-hour lag time.

3.1.3 Uptake from the gastrointestinal tract

No studies have been found in the literature related to the absorption of styrene from the gastrointestinal tract of humans.

Data on the absorption of styrene from the gastrointestinal tract of rats indicate that absorption is rapid and virtually complete. Peak plasma levels were reached in rats within minutes following a dose in aqueous solution, and in less than 2 hours after a dose in oil (244). Almost complete absorption of oral doses of styrene is indicated by the recovery of 90-95 % of the dose of ^{14}C-styrene as urinary metabolites (184,204).

3.2 <u>Distribution</u>

Human studies on the distribution of styrene are limited to its quantitative analysis in blood or subcutaneous adipose tissue biopsies from volunteers or workers after inhalation exposure.

The mean concentrations of styrene in adipose tissue samples were ~3.6 mg/kg (~8 % of total absorption) throughout the first 24 hours after the end of exposure of subjects to 50 ppm styrene during four 30-minute periods of rest and exercise (63). The mean daily uptake of three workers exposed to TWA concentrations of 32-85 mg/m^3 was 193-558 mg and the concentration of styrene in the adipose tissue varied from 2.8 to 11.6 mg/kg (64). Wolff et al. (246) detected styrene in fat biopsy samples from 13 out of 25 styrene polymerization workers.

Animal studies indicate that the distribution of absorbed styrene is rapid. Peak tissue concentrations were reached within 2-4 hours after oral exposure

(184). Organ/blood concentration ratios changed with dose or exposure level (194,242,243,251). Relative concentrations of styrene increased in the liver and brain with increased exposure (243). Relatively high concentrations of styrene have been found in adipose tissue (15,30,220,221,243). Fat has been proposed as the peripheral compartment of a two-compartment toxicokinetic model for styrene (194,251). Blood concentrations are linearly related to the inhaled exposure concentrations below 200 ppm according to a physiologically based pharmacokinetic model (192).

3.3 Biotransformation

The major site of styrene metabolism is the liver. Figure 3 shows the main metabolic pathways of styrene. The initial conversion of styrene into styrene-7,8-oxide is catalyzed by the microsomal cytochrome P450-dependent mono-oxygenases (121). Styrene-7,8-oxide is a reactive intermediate, which can bind covalently to cellular macromolecules (27,88,230).

Microsomal epoxide hydratase catalyzes the conversion of styrene-7,8-oxide into styrene glycol, which is further metabolized into the urinary metabolites mandelic, phenylglyoxylic, benzoic or hippuric acid (120,173). Mandelic acid and phenylglyoxylic acid have been identified as the major urinary metabolites of styrene in humans (10,79,80, 81,252). The product balance of uptake and excretion after an 8-h exposure to 100 ppm of styrene has been shown to be the following: exhaled styrene 2.6 % of the uptake; mandelic acid in urine 56.9 %; phenylglyoxylic acid in urine 33.0 %; hippuric acid in urine 7.5 % (80). The mandelic acid formed from styrene is racemic, having both R- and S-enantiomers (58,117).

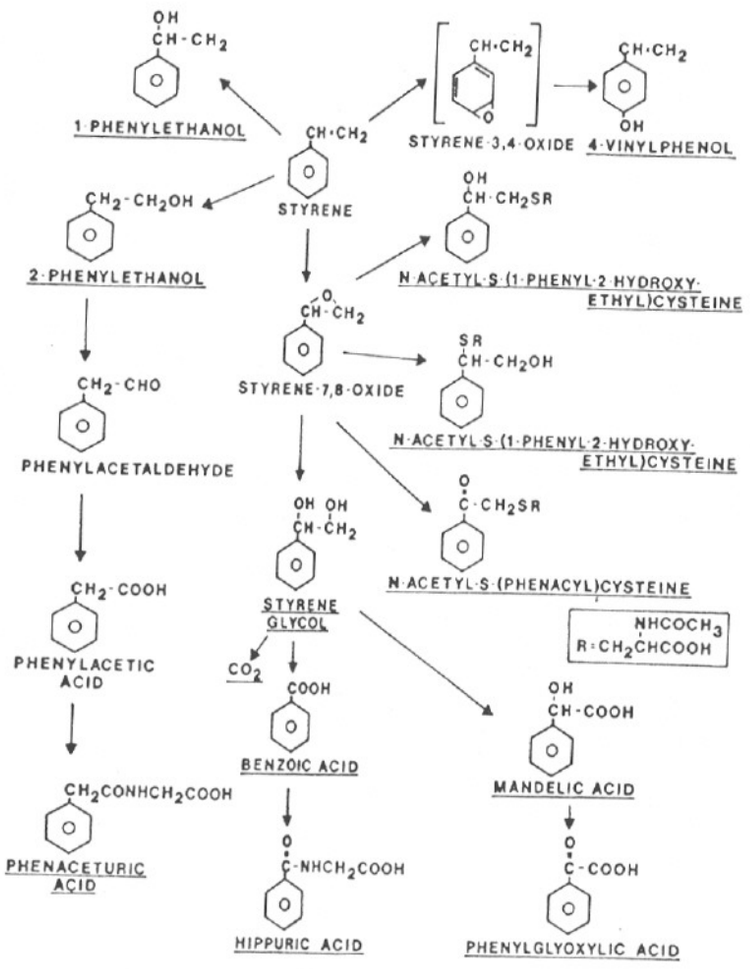


Figure 3. Main metabolic pathways of styrene. Adapted from: Löf (137)

The other detoxification pathway involves the enzymic conjugation of styrene-7,8-oxide with reduced glutathione (GSH) by cytosolic glutathione-S-transferases followed by further biotransformation into mercapturic acids excreted in urine (50,118,140). The cytosolic glutathione transferase in human liver has been shown to catalyze the conjugation of glutathione with styrene-7,8-oxide (177).

Several other minor pathways have also been proposed leading to the formation of 1-phenyl ethanol, 2-phenyl ethanol, and 4-vinylphenol (108). 4-vinylphenol has been identified in the urine of reinforced plastic workers (182). Styrene-3,4-epoxide, which has been shown to be a bacterial mutagen, is probably the metabolic intermediate (235).

3.4 Elimination

Only a small fraction of styrene is eliminated unchanged in urine (107), and very little styrene is exhaled unchanged (1 to 3%) (218). Styrene is almost completely (90-97 %) eliminated as urinary metabolites (79,193,194). The postexposure biological half-lives of mandelic acid and phenylglyoxylic acid in urine have been measured: Mandelic acid (0-20 h after exposure) 3.9-9.4 h; (> 20 h after exposure) 16.6-26.5 h (65,80). For phenylglyoxylic acid (0-50 h after exposure) the half-life is 10.5 $\pm$ 1.4 h (80); for a period 20-200 h after exposure it is between 21.5-26.7 hr (29). The half-lives are influenced by the duration and intensity of exposure. In adipose tissue, the half-life of styrene has been estimated to be about 2-4 days (63).

Styrene is fairly rapidly eliminated from animal tissues with the exception of adipose tissue, which may be a reservoir for administered styrene. The

concentrations in all tissues tested were < 1 µg/g 24 hours after oral dosing (184). Elimination from fat lags behind that from other tissues (178,220,221). Repeated exposure did not result in styrene accumulation (220,221).

In animals, elimination of styrene into the expired air, and urine, and from the blood is rapid and biphasic log-linear at low doses (15,193,194,204,205, 242,243,244,251). At higher doses, the elimination curves indicate saturation of metabolism or excretion (193,194,204,220,221,251).

3.5 Factors affecting the metabolic model

Styrene metabolism is inhibited by the presence of other solvents such as toluene (104,106) or trichloroethylene (104). Mandelic acid excretion in urine was temporarily inhibited by ethanol ingestion in humans exposed to styrene vapors (241). Berode et al. (17) have confirmed the great influence of alcohol intake on mandelic acid kinetics and shown that the kinetics of phenylglyoxylic acid is less influenced than that of mandelic acid.

In rats, phenobarbital treatment increases the metabolism of styrene (202). Also food deprivation, carbohydrate restriction and (three-week) ingestion of ethanol (2 g/d) enhanced the metabolism of styrene in rats (162,202,203). In contrast, 3-methylcholanthrene administration had no effect on the metabolism of styrene.

3.6 Biological monitoring

Styrene has been measured in blood, exhaled air, and

subcutaneous fat. The concentration of styrene in blood and exhaled air falls rapidly during the first hour following termination of exposure.

Gas chromatography is the method of choice for the rapid determination of styrene concentrations in exhaled air (67). The detection limit of the method is 0.04 mg/m^3 (0.01 ppm). Brooks et al. (22) have developed an improved method with increased stability of samples; they were able to reduce the detection limit to 0.02 mg/m^3 (0.005 ppm).

Styrene concentrations in venous blood have been determined by gas chromatography using the "head space" technique (18,242,252) or after previous extraction (22,218) by a direct spectrofluorometric method with a very low reported detection limit (247,248).

The estimation of styrene concentrations in adipose tissue is possible using a needle biopsy technique to obtain the tissue sample. Styrene in the adipose tissue can be determined with gas chromatographic methods (63,208,246). The detection limit for a 10 mg sample is 40 µg styrene/kg of adipose tissue (246).

Evaluation of styrene exposure may be based on the urine concentration of phenylglyoxylic acid (82,94,173), or of mandelic acid (10), on the combined concentrations of mandelic and phenylglyoxylic acids (62,79), or on the ratio of mandelic to phenylglyoxylic acid (173,183). Most of the methods for the determination of mandelic and phenylglyoxylic acid in urine are based on gas chromatographic techniques (24,66,69,78,215). Detection limits ranging from 0.6 to 6 mg/l and variation coefficients ranging from 1 to 3 % have been reported for the different gas chromatographic procedures (66,69,215).

A very promising future method for determining whether exposure to styrene has occurred is to determine the extent of hemoglobin adduct formation of

styrene metabolites following exposure. Byfält-Nordqvist et al. (27) have measured hemoglobin adducts in mice after intraperitoneal administration of styrene oxide. The increase in the formation of hemoglobin adducts was dose-dependent. Hemoglobin adducts accumulate over exposure time and tend to reach steady state levels in chronic exposure, thus giving ideal opportunities for biological monitoring of exposure.

4 GENERAL TOXICOLOGY

4.1 Toxicological mechanisms

Styrene is oxidized to a number of epoxide intermediates by cytochrome P450 mediated oxidases. A suggested mechanism of styrene toxicity is covalent binding of styrene-7,8-oxide to cellular macromolecules e.g. DNA and proteins (27,88,119,230). Induction of mixed-function oxidases (46,47,217,227), epoxide hydrolase activity (179,199,200) and inhibition of glutathione-S-transferase activity (46-48,217) have been observed following styrene exposure. Biochemical changes in the brain in response to styrene exposure have been noted by Agrawal et al. (1), Husain et al. (93), Savolainen and Pfäffli (208), see section 5.8. and Rosengren and Haglid (197).

It has been shown in a cell-free system that styrene oxide modifies deoxynucleosides, aminoacids, human serum proteins, hemoglobin and single and double stranded DNA (88,152,206,231). Hemminki (88) found that the major reaction product of styrene oxide and human hemoglobin was a styrene oxide-cysteine adduct. Byfält-Nordqvist et al. (27) have shown a dose-dependent

increase in the formation of hemoglobin adducts in mice administered styrene or styrene oxide intraperitoneally.

In vitro studies using cells in culture or isolated DNA have generally failed to demonstrate an interaction of styrene oxide with DNA (57). Covalent binding of ^{14}C-styrene and ^{3}H-styrene-7,8-oxide to DNA of various tissues has been demonstrated by Byfält-Nordqvist et al. (27) following ip. injection in male NMRI mice. Levels of binding to guanine in the liver ranged from 17 to 31 nmol/g DNA within 2 h after dosing.

4.2 Acute toxicity

Results of controlled experiments in human volunteers indicated that styrene administered by inhalation at relatively high doses affects the central nervous system (CNS). Drowsiness, listlessness and an altered sense of balance were reported during 4-hour exposure of two male subjects to styrene of 800 ppm (3400 mg/m^3) (31).

Single exposures to 1300 ppm styrene in rats and guinea pigs caused depression, which included weakness and unsteadiness. After a 2500 ppm styrene exposure (10 h), unconsciousness occurred in both animal species. Exposures of 5000 to 10 000 ppm of styrene caused unconsciousness and fatalities in animals. The principal pathological findings in rats and guinea pigs exposed to styrene were severe pulmonary irritation, congestion, edema, hemorrhage and leukocytic infiltration (216).

Wolf et al. (245) reported an acute oral LD_{50} of ~5000 mg/kg for rats treated by gavage. The highest single oral dose resulting in no mortality was 1600 mg/kg, while the lowest dose resulting in 100 % mortality was 8000 mg/kg (216). No mortality was seen in rabbits following dermal applications of styrene of $\leq$ 20.000 mg/kg for 24 hours (85). LC_{50} values ranging

from 2700 ppm for 4 hours (110) to 4620 ppm for 6 hours (19) have been reported from rat inhalation studies. Reported LC_{50} values for mice were 4930 ppm for 4 hours (213) and 2430 ppm for 6 hours (19).

4.3 Subchronic and chronic toxicity

Spencer et al. (216) found no apparent toxic effects in rats after oral exposure to styrene at doses of 100 mg/kg, 5 days/week for 4 weeks. Reduced weight gain was observed at 500 mg/kg and a few deaths were reported at $\geq$ 1000 mg/kg. No histopathologic or hematologic effects were noted in rats at oral doses $\leq$ 667 mg/kg for 6 months (5 days/week); kidney, liver and body weight changes were observed at $\geq$ 400 mg/kg (but not at < 133 mg/kg) (245). Severe toxic effects (mortality, liver necrosis, severe lung congestion) were observed in mice after weekly oral doses of 1350 mg/kg for < 16 weeks (187). Forestomach and kidney lesions were common (incidences not given) in a study of chronic exposure of rats dosed orally with 500 mg/kg/week (187).

5 ORGAN EFFECTS

5.1 Effects on skin, mucous membranes and eyes

After inhalation exposure to 376 ppm of styrene for 1 hour in a controlled experiment with human volunteers, eye and throat irritation occurred (218). Nasal irritation was observed after 20-minute exposures to 920 mg/m^3 (216 ppm), while no signs of toxicity were noted at exposure to 500 mg/m^3 (117 ppm) for 2 hours

(218). Eye and throat irritation was observed at a concentration of 800 ppm for 4 hours (31). In a case-control study of laminating workers, mucous membrane irritation was reported significantly ($P < 0.01$) more often in the exposed group than in the unexposed group (95).

In rats and guinea pigs, eye and nose irritation were noted at levels of 650 ppm, as evidenced by lacrimation, salivation and nasal discharge (216).

5.2 Respiratory effects

There have been several reports concerning the irritation of respiratory mucosa, including the nasal cavity, of workers exposed to styrene vapor. Rowe et al. (198) reported that exposure to less than 1700 mg/m^3 (400 ppm) of styrene did not induce marked symptoms in the nose. There were significant differences between higher (20-85 mg/m^3, 5-20 ppm) and lower (< 4 mg/m^3, 1 ppm) exposure groups with respect to acute lower respiratory symptoms (134,135). No marked differences in lung function test results were observed in styrene-exposed workers (9,95). Obstructive lung changes in 4/21 styrene-exposed workers is reported but no comparison was made with controls (39).

The frequency of lung obstruction was significantly ($P < 0.05$) higher among styrene-methylmethacrylate workers (45.5 %) than in the reference group (18.0 %) (112). The prevalence of chronic bronchitis and asthmatic symptoms were similar between the exposed and control group. In rats, epithelial changes occurred in the nose and trachea after exposure to 800 ppm of styrene for 4 h/d for 8 weeks. The animals were killed three weeks after cessation of exposure. The changes

included vacuolation of epithelial cells, nuclear pyknosis, and "fall-out" of epithelial cells (172). Changes in the nasal mucosa occurred at exposure levels as low as 50 ppm. At 1-d after the last exposure to 150 and 1000 ppm of styrene for three weeks (4h/d, 5d/week), there was a dose-dependent decrease in tracheal and nasal ciliary activity (172). Complete morphological recovery of the nasal and tracheal mucosa occurred at the 12th week post-exposure to 150 ppm styrene. After exposure to 1000 ppm of styrene, the nasal mucosa displayed altered morphology even 12 weeks after exposure.

5.3 Gastrointestinal effects

Basirov (11) reported studies on the digestive system of 130 workers (89 men, 41 women) engaged in styrene-butadiene synthetic rubber manufacture. Average styrene concentrations were 60-130 mg/m^3 (14-31 ppm). Butadiene concentrations were not given. Tests of secretory, excretory, motor, and pepsinogen-generating functions of the stomach were conducted on 20 unexposed people and on 80 exposed workers who had developed symptoms related to the digestive system after working in the plant. Thirty-six had decreased digestive function, 25 had decreased peristalsis, and 51 had decreased acidity. In further examinations, chronic gastritis was diagnosed in 35 of these workers.

5.4 Hepatic effects

The activities of serum enzymes (alanine aminotransferase, ALAT; aspartate aminotransferase, ASAT; and gamma glutamyl transferase, GGT), which are

indications of hepatotoxic effects, were reported normal in 101 styrene workers exposed to an average of 100-300 mg/m^3 (23-70 ppm) (37) and were not significantly different from controls in 84 styrene workers generally exposed to <4 mg/m^3 (< 1 ppm) (222). However, significantly higher levels of ALAT and ASAT were found in workers with exposure to < 100 ppm styrene than in the controls (9). A group of workers exposed to 5-20 ppm of styrene had a significantly higher prevalence of abnormal GGT than a group exposed to < 1 ppm (134,135). Individuals exposed to 50-100 ppm tended to have higher serum enzyme activities than individuals with exposure to < 50 ppm (92).

In Finland, a group of 34 styrene-exposed and 34 reference female workers did not show different ASAT, ALAT and GGT values when followed prospectively for a year. The styrene exposure levels were around 130-170 mg/m^3 (30-40 ppm). Also cholic acid and chenodeoxycholic acid concentrations were of the same magnitude in both groups (97). However, in a study on 23 styrene exposed workers in Sweden, eleven had raised concentrations of either cholic acid or chenodeoxycholic acid or both (60). The exposure levels ranged from 40 mg/m^3 to about 200 mg/m^3.

Changes in hepatic enzyme activity following oral exposure of animals to styrene have been studied by a number of investigators. An increase in mixed function oxidase activity has been reported in rat liver after seven oral doses of 450 or 900 mg/kg/day, but not 270 mg/kg/day (47) and after oral doses of 200 or 400 mg/kg 6 days/week for 100 days (217). A dose-related decrease in hepatic glutathione-S-transferase activity was observed after oral dosing with 200 or 400 mg/kg 6 days/week for 100 days (217), while both hepatic and renal glutathione-S-transferase activity decreased significantly after 450 or 900 mg/kg (but not 270 mg/kg)

oral doses for 7 days (47).

Minor hepatotoxic effects of oral exposure to styrene have been indicated. Elevated levels of ALAT and ASAT were found, and areas of focal necrosis were present in rat liver at a dose level of 400 mg/kg 6 days/week for 100 days (217). Minimal histopathological changes were found in dogs following subchronic oral exposure to styrene (600 mg/kg for 560 days) (189,190). In a study of chronic exposure, small necrotic foci were observed in the liver of rats dying at ~1 year of age; these changes were not observed in rats dying later (187).

The results of inhalation studies of styrene support the oral exposure results. Alterations in hepatic enzyme activity were observed during the 11-week exposure of rats to 300 ppm styrene (6 hours/day, 5 days/week) (227) and after 7 days of exposure to 1900 mg/m^3 (450 ppm) 8 hours/day (199, 200). Minor histological liver alterations were found after < 2 weeks of exposure to 1300 mg/m^3 (300 ppm), 6 hours/day, 5 days/week (227).

The substrate affinity of several hepatic enzymes was altered following intraperitoneal doses of styrene (118). Glutathione depletion was observed in the liver after acute styrene exposure of laboratory animals (47,180,228). Glutathione depletion also occurred following oral dosing of rats with 900 mg/kg (but not 450 mg/kg) for 7 days (47), and following subchronic inhalation exposure (227).

5.5 Renal effects

A series of reports summarized the renal function of workers occupationally exposed to alkyl benzenes (5-8).

The mean urinary concentration of albumin was

significantly higher in the styrene-exposed workers than in controls, but indicators of renal tubular damage were negative (5-6) and the glomerular filtration rate was apparently not affected by styrene exposure (7).

Franchini et al. (72) found increased excretion of albumin and beta-2-mikroglobulin in urine of styrene-exposed (exposure levels about 50 ppm; mean duration of employment 6.4 years) male workers. In contrast, Vyskocil and coworkers (232) were unable to find any difference in the urinary excretion of various proteins between the female workers exposed to styrene (mean level of exposure about 225 mg/m^3 (50 ppm), mean duration of exposure 11 years) as compared to their female unexposed controls.

Urinary excretion of erythrocytes and leukocytes was not reported specifically for styrene workers (8). Alkyl benzene-exposed workers excreted significantly ($P < 0.02$) more erythrocytes and leukocytes than did the controls. The differences between the styrene workers and the other organic solvent workers were reported to be not significant.

No significant difference was found in the renal concentrating ability of the organic solvent workers nor in the glomerular filtration rate of styrene workers compared with controls (5,7).

The glutathione conjugation of styrene gives rise to nephrotoxic mercapturates which inhibit the organic anion transport by renal tubuli (76).

5.6 Hematologic effects

Some authors have reported an increased prevalence of leukopenia, sometimes in association with other hematologic changes (e.g. slight anemia) among workers

exposed to styrene. Other reports have not disclosed any hematologic disorder in men exposed to styrene (175). A significantly reduced platelet count was noted in a group of styrene workers exposed to ~70 ppm for an average of 10 years compared with workers exposed for an average of 1 year (40). A number of other hematology parameters differed from those of a control group. A 30% increase in the number of peripheral monocytes was observed among 20 glass-reinforced plastics workers exposed to styrene (mean concentration 56 mg/m^3) as compared to 22 unexposed referents (83). Relative lymphocytosis was diagnosed in 26 % (88/342) of the styrene/polystyrene production workers (134,135). The incidence of lymphocytosis increased with duration of exposure in the highest exposure subgroup (5-20 ppm). Several other studies have reported results of hematology analyses in styrene workers (32,222) but low exposure levels and lack of comparison with controls makes interpretation of their results difficult.

5.7 Cardiovascular effects

Thiess and Friedheim (222) did not observe any "gross pathological indications" in the electrocardiograms of 84 workers exposed to styrene for 1-36 years.

5.8 Nervous system effects

A significant increase in abnormal EEGs in styrene-exposed workers who show behavioral symptoms is reported (211). A marked change in the proportion of abnormal and normal EEGs occurred when the mandelic acid concentration was >700 mg/l (~31 ppm 8-hour TWA)

(98). In a subgroup of this population, nerve conduction measurements were similar for exposed and control groups. Signs of sensory neuropathy (alterations in amplitude and duration of sensory action potentials) is observed in three groups of workers exposed to < 5, 47 or 125 ppm styrene (196). Slowed radial and peroneal nerve conduction velocities have been found in tested workers (125). Evidence supports nervous system effects in humans because the association increases with higher exposure and longer duration, and correlates with high mandelic acid concentrations in urine.

Several studies have dealt with the reaction times of workers occupationally exposed to styrene. One study (82) in which 17 men were exposed to a styrene concentration of 630 mg/m^3 (150 ppm) showed prolonged simple reaction times in the styrene-exposed workers, both in the morning and in the afternoon, compared with an age-matched control group. A second study of 106 workers in four work places indicated longer and more irregular reaction times in workers exposed to styrene than in controls (74). The differences were still present after a night's rest. The mean styrene concentration determined by continuous measurement in the workers' breathing zone was 57-426 mg/m^3 (13.6-101.4 ppm). The mean duration of exposure was 2.7 years (range 0.1-11.0 years).

Another study with a similar study design also revealed prolonged reaction times during the working day among styrene- and acetone-exposed boat manufacturers (116). The exposed group (7 workers, average styrene concentration 37 mg/m^3 (9 ppm), acetone concentration 82 mg/m^3, mean employment time 10.5 years) did not show any deterioration in sensory motor functions in relation to styrene exposure and the addition task. There was a correlation between the

reaction-time impairment and the total uptake of styrene divided by the estimated amount of adipose tissue.

In two studies (61,139) simple reaction times were measured after exposure to low levels of styrene (below 110 mg/m^3). In a study of female workers, slowed reaction times were found after exposure (139), but not in an other study with exposed male workers (61).

Cherry et al. (36) studied 27 workers (mean age, 23 years) who were exposed to a time-weighted average level of styrene of 386 mg/m^3 (92 ppm). Psychological and behavioral tests revealed, differences only in reaction times between the exposed and unexposed groups. After including new factories and workers to the study (34,35), the authors reported only mood changes related to styrene exposure.

Flodin et al. (70) studied 21 men (mean age 37 years) who had worked in a plant manufacturing reinforced polyester-boats. When the men were examined for the first time, they all were employed at the plant but had been out of exposure for one week. 19 of the men were reexamined after they had been away from styrene exposure for about 8 months. At the time of the first examination, five of the 21 men were diagnosed as having a neurasthenic syndrome (with symptoms such as abnormal tiredness and forgetfulness). After an exposure-free period of 8 months, the subjective well-being of all the subjects had greatly improved, except for one man who in the first examination was diagnosed as having a psycho-organic syndrome (with pathological findings in psychological function tests). The exposure levels had been around 25-50 mg/m^3 since 1981 (8 h time-weighted average).

Mild sensory nerve conduction velocity deficits were found among styrene exposed boat manufacturing workers in Canada (33). The proportion of workers with

reduced nerve conduction velocity rose from 23 % in those exposed to less than 50 ppm (210 mg/m^3) to 71 % in those exposed to more than 100 ppm (420 mg/m^3). Effects on the central nervous system were also seen; the mean reaction time was reduced in those workers whose urine still contained mandelic acid despite the weekend break from exposure. There was some evidence that both central and peripheral nervous systems recovered when workers were removed from exposure.

In a cross-sectional study of psychological functions of male laminating workers, the strongest correlation was found between visuomotor inaccuracy and the exposure (mandelic acid in urine), while psychomotor performance and vigilance correlated slightly (127).

In an Italian study (156) a neuropsychological test battery was administered to 50 workers exposed to styrene, and to 50 controls matched for sex, intelligence, and age. Mandelic acid (MA) and phenylglyoxylic acid (PGA) were measured as exposure indices in the urine collected on Saturday mornings, just before neuropsychological testing. Exposure-response and exposure-effect relationships were found between the intensity of the exposure as reflected by the sum of MA and PGA and the scores of the neuropsychological tests. Verbal learning skills were significantly impaired in workers with a sum of MA and PGA higher than 150 mmol/mole creatinine, corresponding to styrene airborne concentrations higher than 25 ppm (mean daily exposure). Logical memory and visuo-constructive abilities were shown to be significantly affected in workers with MA and PGA higher than 300 mmol/mole creatinine, corresponding to exposure levels of more than 50 ppm of styrene in air.

Oral administration of styrene (~900 mg/kg) to rats for 15 days resulted in significantly elevated

levels of noradrenalin and serotonin in the brain and depressed activity of monoamine oxidase (93), while increased sensitivity of dopamine receptors was observed at oral doses of 200 and 400 mg/kg for 90 days (1).

Exposure of rats to styrene by inhalation (800, 1000 and 1200 ppm, 14 hours per day for 3 weeks) caused a marked hearing loss at each exposure level, assessed by behavioral (conditioned avoidance) and electrophysiologic (brainstem auditory - evoked response) methods (188).

After inhalation exposure, altered enzyme activities were observed in the brain near the end of 11-week exposure to 300 ppm x 6 hours/day (5 days/week) (208). Glial cells were apparently not affected by similar exposure (207). A transient increase in the motor conduction velocity of the tail nerve of rats was observed at exposures to 300 ppm 6 hours/day, 5 days/week (210). Styrene exposure at 320 ppm for three months induced astroglial alterations in rats, as shown by raised concentrations of glial fibrillary acidic protein in the sensory motor cortex and in the hippocampus (197).

5.9 Endocrinological effects

The serum levels of prolactin (PRL), and human growth hormone (HGH) have been reported to be higher in women exposed to styrene (mean 550 mg/m^3, 130 ppm; range 65-300 ppm) than in age-matched controls (157).

Arfini et al. (4) measured the prolactin (PRL) response to thyrotropin-releasing hormone (TRH) in 16 female styrene-exposed workers and in 16 controls. Only one styrene-exposed worker showed a normal response to TRH, compared to 15 of 16 control subjects. Styrene-

exposed workers showed a much higher response to TRH than did the controls. The median urinary excretion of styrene metabolites was 315 mmol/mol creatinine in spot samples collected 15 hours after the last exposure. The effect of these abnormal neuroendocrinological responses on health is not known, and they certainly warrant further study of e.g., gynecologic disturbances associated with hyperprolactinemia, i.e., oligomenorrhea and reduced fertility.

6 IMMUNOTOXICITY AND ALLERGY

There is very little information on the immunological or allergic effects of styrene. In one immunophoresis study of Chmielewski et al. (38), no dose-related differences were observed in concentrations of serum gamma globulin among workers exposed to different concentrations of styrene.

Two cases of occupational asthma due to styrene have been described (151). The subjects complained of cough, breathlessness and symptoms of asthma when coming in contact with styrene. Inhalation challenge with styrene produced an immediate bronchospastic reaction which was followed by a late cutaneous rash in one of the two cases. These two case reports indicate that styrene can be a primary cause of occupational asthma.

7 GENOTOXIC EFFECTS

7.1 Mutagenicity

In bacterial mutagenicity assays, there was no increase in the number of revertant colonies of Salmonella typhimurium when styrene was assayed in the absence of a mammalian metabolic activating system, but in the presence of a metabolic activating system, both positive and negative results have been reported (see Table 1). The putative reactive metabolite, styrene-7,8-oxide, was mutagenic in S. typhimurium with and without metabolic activation (26,49,52,53,57,75,133,150,234). Watabe et al. (235) reported that another presumed styrene metabolite, styrene-3,4-oxide, was a potent mutagen in Salmonella.

The results of gene mutation tests of styrene in yeasts were negative in a conventional assay in vitro (12,132), but positive responses were observed with Saccharomyces cerevisiae when the yeast cells were in the logarithmic growth phase (49) and when styrene was tested in a host-mediated assay (132). In cultures of Chinese hamster V79 cells, styrene did not induce gene mutations when tested with or without a metabolizing system prepared from rodent liver (13,132), except when metabolic activation was provided by a liver perfusion system (13). Styrene was also positive in a recessive lethal assay using Drosophila melanogaster (56).

Increased incidences of chromosome aberrations as a result of styrene exposure have been reported in Chinese hamster CHL cells in vitro with (but not without) metabolic activation (109,144) and in cultured human lymphocytes (111,128,129,185). Styrene did not increase sister chromatid exchanges (SCEs) in Chinese hamster CHO cells when tested with rat liver S-9 mix,

Table 1. Summary of mutagenicity assays in S. typhimurium

Strain	Experimental procedure[a]	Metabolic activation[b]	Dose (range) moles/plate	Result[c]	Reference
TA 1535, TA 100	ST	No	5×10^{-5}	-	(150)
TA 1535, TA 100	PT	RC	10^{-4}-10^{-9}	(+)	(229)
TA 1535, TA 100	ST, PT	RA, HA	up to 10^{-5}	-	(219)
TA 1535, TA 100	PT	RA	10^{-6}-10^{-9}	+	(53)
TA 1535	PT	MP	10^{-5}-10^{-9}	-	(133)
TA 100	LP	RM	10^{-6}	+	(236)
TA 1535, TA 100	PT	RA, RC	10^{-6}-10^{-9}	-	(26)
TA 1535	PT	RA	10^{-5}	+	(186)
TA 1535, TA 100	PT	RA	gaseous	+	(52)
TA 1535, TA 100	PT	RA	up to 5×10^{-6}	-	(49)
TA 1535, TA 100	PT, LP	R, M, H, RA, MA, HA	10^{-6}-10^{-9}	-	(59)
TA 100	PT	RA	10^{-6}-10^{-7}	-	(20)

a) ST, spot test; PT, plate incorporation assay; LP, liquid preincubation assay

b) RC, Clophen-induced rat liver S-9; RA, Aroclor-induced rat liver S-9; RM, 3-methylcholanthrene-induced rat liver S-9; HA, Aroclor-induced hamster liver S-9; R, rat liver S-9; M, mouse liver S-9; H, hamster liver S-9; MP, mouse-phenobarbital induced liver S-9

c) +, positive; (+), slight positive; -, negative

although a positive result has been observed when erythrocytes were used for styrene activation (54,166,167). Styrene also induced SCEs in human whole blood lymphocyte cultures (165,170).

In one study (148) inhalation exposure of rats to styrene resulted in an increase of chromosome aberrations in rat bone marrow, whereas another study gave negative results (214). Styrene was reported to induce SCEs and micronuclei in mice after intraperitoneal (i.p.) injection (41-43,168,212). On the other hand, no effects were found in studies of chromosome aberrations in mice after i.p. or oral treatment with styrene (133,209,212). Neither did styrene induce micronuclei in Chinese hamsters (181).

A slight increase in single strand breaks of DNA in peripheral lymphocytes of styrene exposed workers has been reported (229). A correlation was found between single-strand breaks, urinary excretion of mandelic acid and the concentration of styrene glycol in blood.

Walles and Orsen (233) have shown that styrene can induce single-strand breaks in DNA from styrene-exposed mice. Male mice were injected intraperitoneally with doses of styrene between 1.7 and 10.1 mmol/kg. There were increases in single-strand breaks in DNA from the kidneys of mice 1 h after dosing, which decreased back to the control level by 24 hours. There was also a measurable increase in single-strand breaks in the DNA from brain and lung, but little effect on the DNA from testes. A dose-response effect was demonstrated with DNA from kidney.

7.2 Occupational studies of cytogenetic changes

Several studies show an increase in chromosome aberrations (CA), sister chromatid exchanges (SCE)

and/or micronuclei (MN) in reinforced plastics workers exposed to styrene (3,28,77,84,100,101,147,149,158,159, 163,223) (see Table 2). Most of these studies deal with polyester processing. High average concentrations of styrene in the workplace, up to 1260 mg/m^3 (300 ppm), have been measured in these operations.

Mäki-Paakkanen et al. (158,159) observed an increase in chromosome aberrations (CA) in non-smoking workers exposed (according to urinary mandelic acid values) to about 300 mg/m^3 (70 ppm) of styrene but not in workers exposed to an average concentration of 50 mg/m^3 (10 ppm). Högstedt et al. (101) have observed low concentrations of styrene, average 55 mg/m^3 (13 ppm), to cause micronuclei in human peripheral blood lymphocytes. However, in a recent study on workers exposed to styrene (mean concentration 56 mg/m^3) no increase in micronucleus frequencies or chromosome aberrations were observed (83).

The studies published so far do not show a clear correlation between exposure level and the number of chromosome changes found. Some dose-effect relationship between aberration and SCE induction and the level of exposure to styrene, however, does exist. Camurri et al. (28) reported that the mean aberration values correlated to the level of exposure among styrene-exposed workers (exposure averages of the plants studied ranged from 30-> 400 mg/m^3 or 7-96 ppm). Anderson et al. (3) studied two worker groups with different exposure to styrene. The mean aberration levels of the two groups did not differ, but the authors observed that the personal aberration rate and the amount of exposure to styrene correlated positively in the group with the lower-level exposure to styrene (average of an 8-h workshift multiplied by the number of years of employment 137 mg/m^3 or 32 ppm). Yager et al. (249) have reported a significant increase of SCE

Table 2. Human cytogenetic surveillance studies on blood lymphocytes of workers exposed to styrene

Level of exposure				Mean number of years exposed	Results of cytogenetic studies			Reference
mg/m^3		ppm			Chromosome aberrations	SCEs	Micronuclei	
Mean	Range	Mean	Range					
-	up to 1260			3	+	ND	+	(146)
				8	+	-	ND	(149)
160	60-310	38	14-73	4	+	ND	ND	(100)
197	0-1008	46	0-230	5	+	+	ND	(3)
	30->400		7->92	9	+	+	ND	(28)
	3-758		0.7-178	14	+	ND	ND	(169,223,224)
200	4-900		1-211	4	-	-	ND	(238)
55	4-150	13	1-36	8	ND	ND	+	(101)
190		45		9	-	-	ND	(237)
56	8-190	13.2	2-44		+	-	ND	(84)
102		24		11	-	ND	+	(163)
98	34-263	23	8-61	8	-	-	-	(159)
56	4-164			8	-	ND	-	(83)
64	-			-	-	+	-	(249)

+, positive; -, negative; ND, no data

in workers exposed to styrene concentrations as low as 64 mg/m^3 (15 ppm) in the air. Grummt and Grummt (77) also observed an increase in chromosome aberrations which in peripheral lymphocytes depended on the concentration of styrene in the workplace.

8 CARCINOGENICITY

8.1 Carcinogenicity in humans

Retrospective cohort mortality and case-control studies have been conducted on workers exposed to styrene in the styrene-polystyrene manufacturing industry and in the styrene-butadiene synthetic rubber industry to assess the carcinogenicity of styrene (103). Three studies have suggested an association between leukemia and lymphomas and exposure to styrene.

In a mortality analysis of 2904 US workers in the styrene-polystyrene manufacturing industry exposed to low or moderate levels of styrene (not exceeding 100 ppm), six cases of leukemia (3.4 expected; SMR 176) and seven cases of lymphoma (5.3 expected; SMR 132) were observed. When the incidence was analyzed, seven cases of lymphatic leukaemia (1.6 expected) four cases of all other leukaemias (2.9 expected) and four cases of multiple myeloma (1.6 expected) were found. However, a subset of the cohort had also been exposed to benzene in the past (176).

In a cohort of 622 men exposed for at least one year in the production, polymerization and processing of styrene in the UK, three deaths from non-Hodgkin's lymphoma were found (0.6 expected). Two of them occurred in the age group of 15-44 years (0.3

expected). A cancer incidence study of the same group revealed a further case of lymphatic leukaemia (0.2 expected), and three cases of laryngeal cancer (0.5 expected). The men with lymphoma and leukaemia had had potential exposure to other agents as well (such as acrylonitrile, benzene, ethylene oxide), but styrene was the main agent to which they were exposed (89).

A slight excess of cancers of the lymphatic and hematopoietic tissues (SMR 155) was found in a US cohort of 1662 men employed for at least six months in styrene-butadiene rubber production. A subset of workers employed in the early 1940s had an SMR of 212 (9 observed, 4.3 expected); for leukemias alone, the SMR was 278 (5 observed, 1.8 expected). The mean levels of exposure to styrene had been approximately 1-2 ppm according to measurements carried out at the end of the follow-up. This level, however, was probably not representative of that for the whole period. Concomitant exposure to 1,3-butadiene and to low levels of benzene renders it difficult to single out styrene as a causative factor (146).

In 1977 a study on styrene-butadiene workers was initiated in eight North American plants. The men employed for more than one year and hired between 1943 to 1977 were followed up to 1979. Analysis was done for the total cohort and for groups by the last job held (142). The cohort was updated with four more years of follow-up and with some modifications of design and analysis methods (143). In this study, a fifty per cent increased risk for all lympho-hematopoietic cancers detected in the production workers was attributable to leukemias and to "other lymphatic cancers". These workers had an SMR of 260 for "other lymphatic cancers" and an SMR of 660 for leukemias in blacks.

A nested case-control study within a cohort of synthetic styrene-butadiene rubber workers has been

carried out on lympho-hematopoietic cancers (201). The work-time exposure histories of all the incident lympho-hematopoietic cancers were contrasted to those of the controls. Matching was done by plant, age, hiring date, duration of work and survival up to the date of death of the case. Exposures were estimated through a ranked job exposure matrix. The analysis of 59 cases and 193 controls indicated a two-fold lympho-hematopoietic cancer risk. The workers exposed to butadiene had a six to eight-fold increased risk of leukemia. The observed association of styrene with leukemia, however, disappeared when it was adjusted for butadiene exposure. A positive trend of increasing leukemia risk with increasing styrene exposure was observed. The risk associated with styrene, when adjusted for butadiene, increased to an odds ratio of 2.9 in the ten year-lag and of 2.4 in the 20-year lag.

There was a statistically nonsignificant 60 to 70 per cent increased risk for "other lymphatic cancers" in the workers exposed to 1,3-butadiene, whereas for styrene it was 20 to 30 per cent.

In the study by Santos-Burgoa (201), workers with high butadiene exposure and low styrene exposure had a significant seven-fold risk for "other lymphatic cancers"; when the worker's exposure to styrene is high but to butadiene low, he has a (non-significant) risk of the same magnitude.

A UK cohort study of 7949 men and women employed during 1947-1984 in eight companies manufacturing glass-reinforced plastics involving high exposure to styrene showed no excess mortality from cancer (188 observed, 233.7 expected). There was a deficit of deaths from lymphoid and hematopoietic cancer (6 observed, 14.9 expected). An additional eight cases of lymphoma and leukemia occurred in workers still alive or who had died from other causes. There was a slight

excess of lung cancer (89 observed, 80.1 expected). An analysis by level of exposure gave some indication of a dose-response relationship, but there was no clear relationship with time since first exposure (45).

Two cohort studies showed no excess of lymphoma or leukemia, or any other cancer (99,174). Both of these studies had low statistical power because the cohorts were young and there had been only a short follow-up since the commencement of exposure.

In a case-referent study, designed to investigate a possible connection between background radiation and acute myeloid leukemia, three cases out of 59 and one referent out of 354 reported past exposure to styrene (rate-ratio, 18.9; 95 % confidence interval, 1.9-357) (71).

8.2 Carcinogenicity in animals

Several long-term animal carcinogenicity studies have shown statistically significant increases in the incidences of tumors at some sites in specific test groups following styrene exposure. Increased tumor incidences were observed in four studies (44,113,160, 187).

The studies by Jersey et al. (113) and Conti et al. (44) have indicated that inhalation of styrene causes cancer in laboratory animals. Groups of male or female Sprague-Dawley rats were exposed for 6 hours/day, 5 days/week to styrene vapor at 600 or 1200 ppm (113). The high dose was reduced to 1000 ppm after 2 months. The study was terminated after 24 months. A statistically significant increase in mammary adenocarcinoma was observed in the females exposed to 600 ppm.

Inhalation of styrene has been reported to increase the incidence of total (benign and malignant) mammary

tumors and malignant mammary tumors at all levels of exposure in Sprague-Dawley rats (25 ppm, 50 ppm, 100 ppm, 200 ppm, 300 ppm for 52 weeks) (44). The increase in the incidence of malignant mammary tumors was dose-related. No such increase was observed in rats exposed to styrene by intragastric administration or by ingestion.

Ponomarkov and Tomatis (187) administered styrene in olive oil by gavage to female O_{20} mice, C57Bl mice, and BDIV rats once on the 17th day of gestation and then weekly throughout their offsprings' lifetimes. A statistically significant increased incidence and earlier onset of lung tumors were observed in the O_{20} offspring, but there was a high background tumor rate in this strain. A few rare tumors were observed in the BDIV offspring. In the C57Bl offspring there was a statistically significant increased incidence of liver tumors compared with pooled, untreated and vehicle controls.

In a study carried out by the US National Cancer Institute (1979) styrene was administered at 150 or 300 mg/kg daily in corn oil by gavage to B6C3F1 mice. Exposure was terminated after 78 weeks, and the study was terminated after 91 weeks. Statistically significant increased incidences of lung alveolar/bronchiolar adenomas or carcinomas were observed in both exposed groups with a statistically significant dose-related trend.

In an inadequately reported study in rats, exposure to styrene by inhalation or ingestion was associated with a small, statistically nonsignificant increase in the incidence of tumors of the brain (141). Sprague-Dawley rats were exposed to styrene in olive oil by gavage (0, 50 or 250 mg/kg) or inhalation to styrene vapors (0, 25, 50, 100, 200 or 300 ppm).

9 REPRODUCTION AND TERATOGENICITY

Studies of possible reproductive effects of styrene are limited in number and design (small sample sizes; quality of exposure data). Hemminki et al. (87) found that the rate of spontaneous abortions in Finnish styrene industry workers was higher ($p < 0.01$) than the rate among all Finnish women. The ratio of spontaneous abortions to the number of births was significantly ($p < 0.001$) higher in styrene workers. This excess was not, however, confirmed in a follow-up study (86). An interview study revealed no large differences between 67 controls and 67 lamination workers with styrene exposures estimated as 66 ppm (96).

In a matched case-control study, no increased risk of spontaneous abortions was observed among workers processing polymerized plastics or heated plastics made of styrene in Finland (126). McDonald et al. (145) have reported an elevated ratio (1.58; 90% confidence intevals 1.02-2.35) of observed to expected spontaneous abortions in women whose work included the processing of polystyrene in Canada. The pregnancy outcome study in Sweden and Norway did not reveal any increased occurrence of stillbirths or malformations among women working in the processing of styrene (2). Mothers of 2/43 children born with CNS defects in Finland during a 9-month period were employed in the reinforced plastic industry with exposure to styrene (91). The authors estimated that 12 births would be expected among the styrene exposed workers, so a rate of 2/12 infants with CNS effects was high, compared with the expected rate of 0.5/1000.

Lemasters et al. (123) did not find statistically significant effects on menstrual function in 174 exposed workers compared with 449 controls. A

dose-related trend to low birth weight in infants born to styrene workers did not reach statistical significance in a study of 1050 exposed workers from the same population (124).

No teratogenic effects of styrene have been observed in rats exposed orally (153,154) or in rats, mice, rabbits and hamsters following inhalation exposure (115,153,155,191). The doses or exposure levels were sufficiently high to cause some maternotoxic effects in the oral and inhalation studies conducted by Murray et al. (153-155). Fetotoxicity (increased incidence of resorptions or dead fetuses) was reported by Kankaanpää et al. (115) in mice exposed to 250 ppm 6 hours/day, on days 6-16 of gestation and in hamsters exposed to 1000 ppm 6 hours/day, on days 6-18 of gestation (but not in hamsters exposed to < 750 ppm).

10 RELATION BETWEEN EXPOSURE, EFFECT AND RESPONSE

10.1 Effects of short-term exposure to styrene

The two major effects of short-term exposure to styrene in humans and in laboratory animals include irritation (both skin and respiratory tract) and central nervous system effects. Inhalation exposure to 376 ppm styrene for 1 hour resulted in eye and throat irritation in humans, and in altered neurophysiological function (balance difficulties in Romberg's test) (218). Nasal irritation was observed after 20 min exposure to 216 ppm, while no overt signs of toxicity were noted at exposure to 117 ppm for 2 hours. Reaction time was significantly increased during the last of

four 30-minute exposures to increasing concentrations of styrene (50, 150, 250 and 350 ppm) (73). The results from animal studies are consistent with the reports of acute toxic effects in humans. Morphological changes in the nasal mucosa of rats have occurred at styrene concentrations as low as 50 ppm (171).

10.2 Effects of long-term exposure to styrene

10.2.1 Nervous system effects

Styrene exposure-related effects including prenarcotic symptoms, fatigue and mucous membrane irritation have been reported (95,134,135,196). In comparison with controls, increased reaction times have been observed in workers exposed to styrene at concentrations above 150 ppm (82). This was not observed at lower styrene concentrations. Gamberale et al. (74) noted increased reaction times at exposure levels of 13-101 ppm mean concentrations. Cherry et al. (34) found a correlation between mandelic acid concentration and start-of-shift reaction time. Lindström et al. (127) reported that altered psychological functions (particularly visuomotor accuracy) were related to styrene exposure. The impairment in visuomotor accuracy was evident at urinary mandelic acid concentration of 800 mg/l (corresponds to 36 ppm of styrene, TWA, 8-hour). More pronounced changes in visuomotor and psychomotor performance occurred at urinary mandelic acid concentrations of 1200 mg/l and above (about 55 ppm 8-hour TWA) (98).

Flodin et al. (70) studied the prevalence of neurasthenic symptoms among 21 reinforced plastic workers, both during employment and after a period of 8

months' unemployment. The symptoms of neurasthenia were reported to fade away after 3-6 months of non-exposure. The styrene levels in the plant had been low, around 25-50 mg/m^3 since 1980. These findings contradict those of Edling and Ekberg (61) who did not find any symptoms of neurasthenia at a styrene exposure concentration of 50 mg/m^3. The difference may be explained by the differences in physical work load; the workers studied by Flodin et al. (70) did more physically demanding tasks than the workers examined by Edling and Ekberg (61).

10.2.2 Cytogenetic effects

Several studies have shown that the number of chromosome aberrations, sister-chromatid exchanges, or micronuclei are increased in the peripheral blood lymphocytes of workers after occupational exposure to styrene (cf. Table 2). Most of these studies come from the reinforced plastics industry, where airborne styrene concentrations have been around 25-30 ppm and above. However, dose response relationships have been sought even at lower styrene levels. Camurri et al. (28) found that the average percentage of lymphocytes with aberrations was already increased in workers exposed to around 10 ppm of styrene. Mäki-Paakkanen (159) did not find an increase in the number of chromosome aberrations in men from a plant with a low (around 20 ppm) exposure to styrene. Högstedt et al. (101) have found an increase in the number of micronuclei in lymphocytes at low concentrations of styrene (around 55 mg/m^3, 13 ppm). In a recent study, however, no increase in micronuclei or chromosomal aberrations was observed in workers exposed to similar styrene concentrations (83). In a carefully conducted

study, Yager et al. (249) found an increase in SCEs in workers exposed to low styrene concentrations (around 15 ppm).

10.2.3 Carcinogenic effects

Workers in the styrene-butadiene rubber (SBR) plants have been found in many studies to have elevated ratios for lymphatic and hematopoietic cancer. After the initial case reports NIOSH initiated a retrospective cohort study in two styrene-butadiene plants. The results indicate that the SMR for lymphatic and hematopoietic cancer was above 100 for the group of workers from one of the plants (146). A subgroup from this plant with higher styrene exposure had SMRs of 212 for overall lymphatic and hematopoietic malignancies. Ott et al. (176) and Matanoski et al. (143) also found an elevated risk for lympho-hematopoietic cancer in people exposed in SBR plants. Hodgson and Jones (89) found an association between exposure in styrene production, polymerization and processing and leukemia and/or lymphoma.

The nested case-control study of lympho-hematopoietic malignancies in the SBR industry by Santos-Burgoa (201) indicates a two-fold lympho-hematopoietic cancer risk; the longest employment in the combined categories of operation services, laboratory and utilities had a leukemia odds ratio of six. Santos-Burgoa (201) incriminates butadiene. The lack of environmental data nevertheless makes the assessment of the relative importance of styrene and butadiene difficult, if not impossible.

The International Agency for Research on Cancer (103) has considered styrene as possibly carcinogenic to humans (Group 2B).

10.2.4 Other effects

Some reproductive outcome disturbances have been described among styrene workers; the results are, however, far from conclusive (for discussion, see section 9). The most interesting findings on the impairment of the dopaminergic modulation of pituitary secretion in styrene-exposed workers may explain some of the menstrual and sexual disturbances observed. The neuroendocrine effects of styrene exposure have been observed in concentrations of styrene above 50 ppm (for references, see section 5.9).

11 NEEDS FOR FURTHER RESEARCH

Well conducted human epidemiological studies, with monitored exposure levels, are necessary to evaluate the possible causal role of styrene in the lympho-hematopoietic cancers observed among styrene-butadiene rubber workers. Other populations, not exposed to 1,3-butadiene should be identified and studied further epidemiologically.

To date the evidence for carcinogenicity in experimental animals is inadequate, because of limited data from oral dosing or inhalation studies. There is a clear need for additional toxicological studies, preferably by the inhalation exposure route.

The potential of styrene to interfere with the immunosurveillance should be studied, both in animals and in humans.

The neuroendocrinological effects of styrene should be studied further, especially those related to the hypothalamic functions.

12 DISCUSSION AND EVALUATION

The neuropsychological effects are the critical effects which should be taken into consideration in the establishment of an occupational exposure limit for styrene. The carcinogenic potential of styrene and the genetic (chromosomal) changes induced by styrene are additional issues which should be considered when exposure limits are being set.

Long-term moderate or high level exposure to styrene can cause disturbances in nervous system functions, as evidenced by the increased occurrence of abnormal EEGs, and reduced nerve conduction velocities in occupationally exposed styrene workers. The signs and symptoms of neurotoxicity in exposed people increase with higher exposure and longer durations. CNS functions such as logical memory and visuo-constructive abilities were affected at concentrations above 50 ppm, whereas verbal learning skills were impaired already at concentrations of 25 ppm and above.

Symptoms of neurasthenia have been described in styrene exposure well below 25 ppm in a group of workers with moderate physical work load, but not in a group with light physical load. It is possible, though, that the past exposure levels have in fact been considerably higher than the 25 ppm indicated.

The recent epidemiological studies on styrene-butadiene rubber (SBR) workers suggest an increased lympho-hematopoietic cancer risk. It is, however, difficult at the moment to evaluate the independent role of styrene in the observed association. Workers in the SBR industry are exposed also to other potential carcinogens such as 1,3-butadiene. In the experimental studies, there is some (according to the IARC evaluation, "limited")

evidence that styrene is a carcinogen. The other evidence, increased incidence of chromosomal aberrations in workers exposed to styrene, and the preliminary evidence of the DNA adducts in lymphocytes in styrene-exposed people, supports the interpretation of styrene being a human carcinogen. Using all the evidence available, the overall evaluation stated in IARC Monographs is, that styrene is a possible human carcinogen (103).

Styrene is a well-known experimental clastogenic agent. Workers employed in the reinforced plastics industry, with high exposure to styrene, have been shown in a number of studies from various countries (see Table 2) to have an increased number of chromosomal changes in their lymphocytes. There is no consistent evidence for a dose-response relationship, although increases in chromosome aberrations have been observed in populations exposed in the reinforced plastics industry to styrene usually in excess of 25 ppm.

The difficulty to demonstrate a clear dose-response relationship may depend on factors such as our inability to identify the relevant exposure parameter, the mean level of exposure not being the most sensitive one. High occasional exposure peaks may be of greater importance than the time-weighted average level.

Styrene is oxidized to styrene 7,8-oxide, a mutagen and an animal carcinogen, which is further converted to styrene glycol, mandelic and phenylglyoxylic acid by appropriate enzymes. The suggested mechanism of styrene-induced genotoxicity is the covalent binding of styrene 7,8-oxide to DNA. There is preliminary evidence that this also takes place in humans (130,131). Induction and inhibition of the enzymes responsible for the formation and inactivation of the epoxides can modulate the toxicity of styrene, and this can also be

one source of interindividual variability in susceptibility.

13 SUMMARY

H. Vainio: Styrene. Nordic Expert Group for Documentation of Occupational Exposure Limits. Arbete och Hälsa

In this document relevant data are summarized for the purpose of establishing permissible levels of occupational exposure to styrene. Of the effects described, the neuropsychologic effects of styrene, in conjunction with its cytogenetic effects and the potential carcinogenicity of styrene, should be taken into consideration in the setting of occupational exposure limits.

In English. 252 references.

A Swedish version is available in Arbete och Hälsa 1990:49

Key words: Styrene, occupational exposure limits, neuropsychiatric effects, carcinogenicity, mutagenicity.

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Appendix I. Occupational exposure limits for airborne styrene

Country	mg/m^3	ppm	Year	Note	Ref.
Denmark	105	25	1988		2
Finland	85	20	1987		9
	420	100		15 min.	
France	215	50	1988		10
Island	210	50	1978		7
Netherlands	420	100	1989		6
Norway	105	25	1989		1
Sweden	110	25	1989	S	3
	300	75		STV	
UK	420	100	1987		
	1050	250		10 min.	4
USA(ACGIH)	215	50	1988-89		
	425	100		STEL	8
(OSHA)	215	50	1989		
	425	100		STEL	11
USSR	5		1978	g	5

g = gas
S = skin
STV = short time value
STEL = short-term exposure limit

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WELDING GASES AND FUMES

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Contents

1. Background

Welding can be defined as joining metals with the help of heat, with or without the simultaneous use of pressure. In gas welding and arc welding, the seam is made by heating the basic material at least to the point where it begins to flow (the melting point). Welding can also be done by atomic diffusion (the atomic hydrogen method). Soldering differs from welding in that the solder (the added material) is heated to a working temperature that is lower than the melting point of the basic material (100).

Fusion welding is the most widely used kind of welding, and arc welding is the fusion welding method most common today. This document discusses only methods of metal welding and cutting. It does not take up welding of plastics.

Welders are estimated to make up more than 1% of the workforce in industrialized countries (68). The air contaminants associated with manual welding are thus responsible for a significant amount of occupational exposure.

2. Methods and exposures

There are a large number of methods and variations of methods for fusion welding and related procedures: flame cutting, plasma-arc cutting, and arc-air gouging. The most common manual methods are described here, since they are the ones responsible for the highest average daily exposures to air contaminants.

The exposure descriptions are drawn primarily from a series of studies of nearly 500 welders, made by the authors during the 1970s and summarized in two reports (174, 175).

During welding and similar procedures, air contaminants are produced from four sources: 1. the basic material of the workpiece, 2. the coating on this material, 3. the added material, and 4. the surrounding air.

The basic material consists of metals, which contain alloy elements and contaminants, and may also be coated or plated. The composition of the three most important basic materials -- steel, stainless steel and aluminum -- usually varies within the following limits (w/w):

Steel can contain up to 0.3% chromium, up to 0.4% copper and up to 1% manganese, while stainless steel can contain 10 to 20% chromium, up to 1% manganese, up to 20% nickel and up to 2.7% molybdenum, as well as small amounts of such elements as niobium, titanium, copper and aluminum. Special steels can have even higher proportions of chromium and nickel (174). Steel contains small amounts of carbon, usually less than 1%.

Construction aluminum can contain up to 6% magnesium, up to 0.3% chromium, up to 1.5% manganese, up to 0.8% iron, up to 0.1% copper and up to 5% zinc (174).

In shielded metal-arc welding, the metal core (the weld material) of the electrode has about the same composition as the metal of the workpiece. The composition of the outer layer depends on the type of electrode. For basic electrodes, calcium and fluoride are the dominant components. Rutile electrodes contain almost 50% titanium dioxide by weight, while the coatings of the now

rare acid electrodes contain a lot of quartz (174). Special metals such as barium (32) and zirconium (174) are sometimes used in the coatings.

Organic coatings on the workpiece are not burned away entirely; considerable amounts are broken down and vaporized by the heat in the zone around the weld. Evidence of this is given by the presence of organic substances with low molecular weight in the air around the welding operation, where they can sometimes account for over 10% of total contaminants. In welding experiments using steel coated with a shop primer containing zinc tetraoxychromate as pigment and polyvinyl butyral and phenol resin as binders, the following substances were identified in the air: butanal, hexanal, phenol, cresol and ethoxyethyl acetate, as well as the following organic solvents: butanol, toluene and xylenes (16).

For gas-shielded metal-arc welding (MIG or MAG, cf. below) a melting electrode is used, with approximately the same composition as the basic material. In both MIG/MAG welding and tungsten inert gas arc welding (TIG) the weld seam is often made by added material with the same composition as the workpiece or compatible with it. The gas shield can be pure argon in both cases. Other shield gases can also be used in gas-shielded metal arc welding: argon with a few percent oxygen (MIG welding), a mixture of 80% argon and 20% oxygen, or pure carbon dioxide (MAG welding) (174).

The fourth source of air contaminants, the surrounding air, can contain (in addition to its main components, oxygen and nitrogen) contaminants such as the chlorinated hydrocarbons used for degreasing. When these are exposed to the heat and radiation from welding operations they can form new substances in the air, e.g. nitrogen oxides (heat); ozone (UV radiation) (174); phosgene from perchloroethylene, trichloroethylene and methyl chloroform; and dichloroacetyl chloride from trichloroethylene (5, 29, 30, 63, 142).

The exposure of the individual welder is also affected by the amount of welding done in comparison with other jobs (the arc-time factor, cf. 2.7.1.), the general ventilation, and the use of protective measures, especially local exhaust ventilation.

2.1. Gas welding

2.1.1 Method description

Gas welding is usually done by mixing acetylene and oxygen (O_2) in suitable proportions in a welding torch. The gas mixture is burned, producing a flame with a temperature of about 3100° C. The proportions of oxygen and acetylene used depend on the nature of the job to be done (100).

Little or no oxygen yields a sooty flame, used for igniting the torch. The addition of a bit more oxygen, so that a bright, clear yellow zone is produced in front of the nozzle, yields a carbonizing flame used for welding cast iron, aluminum and lead, as well as for brazing.

As the amount of oxygen is increased the carbon flame fades and then disappears; the "normal flame" is at just this point. It is used for gas welding of steel and copper. The normal flame is reducing, since it takes some of the oxygen necessary for full combustion from the surrounding air. The reducing effect

protects the melted metal from the effects of oxygen, which means that steel can be welded without using a flux.

Further increasing the oxygen and reducing the amount of acetylene yields first a weakly reducing flame, used to weld brass and bronze and for braze welding. An oxidizing flame is obtained by reducing the amount of acetylene and further increasing the oxygen, so that both the central flame and the outer cone are shortened.

The surplus oxygen has an oxidizing effect on the welded material. This method is used for brasses that are difficult to weld (100).

2.1.2. Air contaminants

According to a survey made in 1976, nearly 32,000 people in Sweden worked with welding, but only about 12% of them with gas welding, usually of carbon steel. This helps to explain why exposure studies of air contaminants have been mostly concerned with metal-arc welding. Some of the older measurements (1950 and earlier) of gases, such as nitrogen dioxide around gas welding (4), are clearly incorrect, and are not included here.

Gas welding is not a single method, as can be seen from the above description. There are numerous variations, all of which affect the nature and concentration of the contaminants in the air. Gas welding is done at lower temperatures than metal-arc welding and can be expected to produce less air contaminants than metal-arc welding, particularly when the latter is done with melting electrodes, which are responsible for the largest contribution of air particles.

2.2. Flame cutting and plasma-arc cutting

2.2.1. Method description

In flame cutting, the basic material is heated with a flame, usually oxyacetylene, and when the metal is light red (at ignition temperature) a high-pressure stream of oxygen is directed at the hot metal. When steel is cut, the oxygen oxidizes the iron to magnetite (Fe_3O_4), which has a melting point below that of iron; it therefore melts immediately and is blown away by the stream of oxygen. The process is then maintained by the exothermic oxidation reaction and the steel does not melt (33).

Plasma-arc cutting is done with a plasma created by leading gas through an electric arc, where it is heated to the plasma state. The temperature reaches 10,000 to 30,000° C. This method can be used to cut metals with high melting points, including stainless steel and aluminum. The cutting speed is high (8).

2.2.2. Air contaminants

In flame-cutting experiments (oxyacetylene torch) under workshop conditions, primed and untreated steel plate was cut with a machine. Air samples, taken in the zone where the operator would otherwise be, contained 9 - 37 mg/m^3 (9 - 35 ppm) CO, 0.22 - 1.8 mg/m^3 (0.18 - 1.49 ppm) NO, and 0.04 - 0.64 mg/m^3 (0.02 - 0.30 ppm) NO_2. Concentrations of these gases can be much higher around flame-cutting in confined, badly ventilated spaces. The NO_2 concentration in unventilated work areas can exceed 190 mg/m^3 (100 ppm) (131).

Pyrolysis products from the commonly used shop primers, which are known to be irritative, were found in concentrations that were low in relation to their exposure limits (the highest was a few hundredths of one ppm). They may nevertheless give rise to odor and irritation (176).

In a study of ten shops where plasma-arc cutting was done, eight of which worked with stainless steel and two with aluminum, monitoring measurements made in the operators' breathing zones during cutting of stainless steel showed total particle concentrations of 0.5 to 21 mg/m^3 (8). The wide range is explained by differences in ventilation. Measured concentrations of chromium ranged from 0.18 to 0.35 mg/m^3, with a single reading of 2.6 mg/m^3; nickel ranged from 0.12 to 0.29 mg/m^3, with one peak of 2.2 mg/m^3 (8). Measured nitrogen dioxide concentrations ranged from 0.10 to 4.3 mg/m^3 (0.06 to 2.3 ppm); the geometric mean was 0.9 mg/m^3 (0.5 ppm). Ozone concentrations were extremely low (8).

2.3.Shielded metal-arc welding with coated electrodes

2.3.1.Method description

In shielded metal-arc welding with coated electrodes, or manual metal-arc welding, an electric current flows between the electrode and the workpiece. The arc melts both the electrode and the surfaces to be joined. The electrode, which melts down as welding progresses, is coated (Figure 1). The composition of the coating is designed to facilitate ignition of the arc, stablilize the arc, remove the oxide layer on the workpiece, protect the weld from oxidation and nitrogen absorption, supply the proper alloy material for the weld, and form a slag that shapes and supports the weld. Either alternating or direct current may be used. When the arc is formed, an arc current is created which for coated electrodes is in the range 20 to 40 volts. The current intensity can range from 50 to 400 amperes (174).

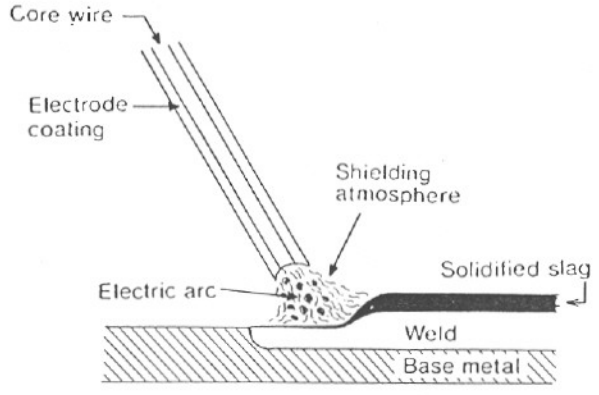

Figure 1. Shielded metal-arc welding

2.3.2. Air contaminants

Ninety welders who used coated electrodes for metal-arc welding of steel with little or no added alloys, and who worked without special ventilation, were studied for exposure to air contaminants. It was found that the time-weighted average of total particles in the breathing zone (inside helmets) in a workday was below about 10 mg/m^3 for half the welders, and below 17 mg/m^3 for 75% of them. When local exhaust equipment was used, these values dropped to 3 mg/m^3 and 7 mg/m^3 (174, 175).

In a similar study of 86 welders who used coated electrodes for metal-arc welding of stainless steel and who worked without local exhaust equipment, the time-weighted average of total particles in the breathing zone (inside helmets) in one workday was below 5 mg/m^3 for half of them, and below about 7 mg/m^3 for 75% of them. The concentration of chromium (as CrO_3) in inhaled air was below 2 mg/m^3 for half of them, and below 4 mg/m^3 for 75% of them (174, 175).

Welding with nickel electrodes (75% nickel) yielded an average 0.4 mg nickel/m^3 (0.07 - 1.1 mg/m^3) (189), but no nickel carbonyl was found (64).

Nitrogen oxide and ozone are the gases most relevant to occupational hygiene. Ozone concentrations around metal-arc welding with coated electrodes are very low, about equal to background values (174, 175).

With welding of steel and stainless steel, half the short-term values for total nitrogen oxides (about 50% each of nitrogen monoxide and nitrogen dioxide), in this case immediately outside the face-guard, were below 0.8 mg/m^3 (0.5 ppm). With welding in stainless steel, values above 5 mg/m^3 (3 ppm) were found in about 10% of the cases (174, 175).

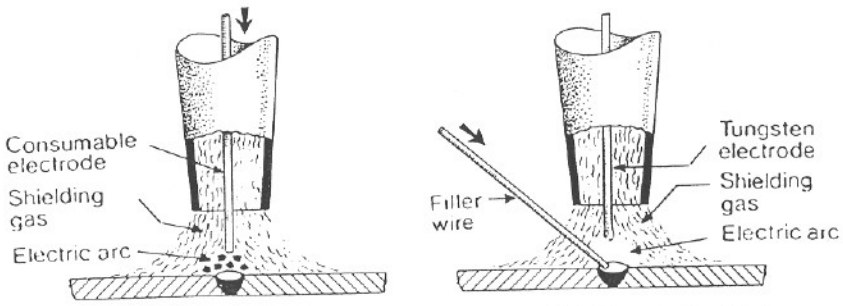

Figure 2. Gas-shielded arc welding, MIG (Metal Inert Gas) and TIG (Tungsten Inert Gas)

2.4. Gas-shielded metal-arc welding

2.4.1. Method description

In gas-shielded metal-arc welding, a protective gas is used to shield the seam from the harmful effects of air (Figure 2). The method was developed during the 1940s and 1950s for welding of aluminum and aluminum alloys, and has since been adapted to other materials.

The method has two main variations. In the first, the weld is made with a melting electrode, which is fed forward as it is used: MIG (Metal Inert Gas) or MAG (Metal Active Gas) welding. Direct current is used in both cases.

MIG and MAG welding are done either as short-arc welding or as spray-arc welding, depending mostly on the thickness of the plate. In short-arc welding (arc length about 2 mm) a metal drop is formed on the tip of the electrode, short-circuits the arc and is then broken off, providing material for the weld. After the short circuit the arc re-forms. This cycle recurs about 200 times per second and is accompanied by a characteristic staccato sound. This kind of welding heats the workpiece less than spray-arc welding, and is used to weld thin plate.

Spray-arc welding is done with a longer arc (≤5 mm). The weld material is transported in the arc. Both the voltage and the amperage are higher, so more heat is produced.

The second type of gas-shielded metal-arc welding, TIG (Tungsten Inert Gas) welding, is done with a non-melting electrode. Alternating current is used in TIG welding in order to break down the oxides without heating the electrode too much.

The arc is ignited with high-frequency alternating current, which is usually turned on constantly during AC welding to re-ignite the arc when the polarity alternates. Both TIG welding and MIG welding can be done with pulsating (intermittent) current (174, 175).

2.4.2. Air contaminants

Air was monitored in the breathing zone of welders using gas-shielded arc welding (MAG) on unalloyed steel. They were working without local exhaust equipment. The time-weighted average total particle concentration in the breathing zone (inside helmets) over one workday was below 7 mg/m^3 for half the welders, and below 12 mg/m^3 for 75% of them. Values were 5 mg/m^3 and 8 mg/m^3 when local exhaust equipment was used (174, 175). Local exhaust equipment is less effective for gas-shielded arc welding than for metal-arc welding with coated electrodes.

Exposure to air contaminants was monitored for 102 welders who did gas-shielded arc welding (MIG) of aluminum. When local exhaust equipment was not used, the average one-day total particle concentration in the breathing zone (inside helmets) was below 9 mg/m^3 for half of them and below 22 mg/m^3 for 75% of them. When local exhaust equipment was used, these values were about 7 and 12 mg/m^3 (174, 175).

With TIG welding in aluminum, the time-weighted average one-day total particle concentration (inside helmets) was below 1 mg/m^3 for half the welders and below 2 mg/m^3 for 75% of them (174, 175),

These welding methods are associated with exposure to both nitrogen dioxide and ozone. The ozone problem is most pronounced with MIG welding of aluminum. Half of the short-term values for ozone concentrations in the breathing zone were below 0.16 mg/m^3 (0.08 ppm) and 75% were below 0.35 mg/m^3 (0.18 ppm); 90% of short-term values were below 0.82 mg/m^3 (0.42 ppm). With TIG welding in aluminum, 90% of measured ozone concentrations were below 0.16 mg/m^3 (0.08 ppm). About the same relatively low concentrations were measured around various forms of gas-shielded arc welding in steel.

With TIG-welding in aluminium, half of the short-term values for total nitrogen oxides (about 50% nitrogen monoxide and 50% nitrogen dioxide) measured directly outside the helmets were 1.5 mg/m^3 (1 ppm) or below; 75% were below 4.6 mg/m^3 (3 ppm); and 90% were below 11.7 mg/m^3 (7.6 ppm). A few readings above 4.6 mg/m^3 (3 ppm) were also measured in the breathing zone around MIG welding of aluminum. Gas-shielded arc welding of steel and stainless steel were associated with lower concentrations (174, 175).

Electrodes alloyed with 2% thorium are often used for TIG welding of stainless steel. Thorium is a natural alpha radiator; its radiation has been measured at <0. 9 disintegrations/ minute/ m^3 (19), equal to <0.015 becquerel per m^3.

2.5. Arc-air gouging

2.5.1. Method description

In arc-air gouging, an electric arc is formed between the workpiece and an electrode. The electrode consists of a carbon rod, which is frequently coated with copper (Figure 3). The basic material melts and is blown away by compressed air. This method is useful for preparing seams and removing faulty welds.

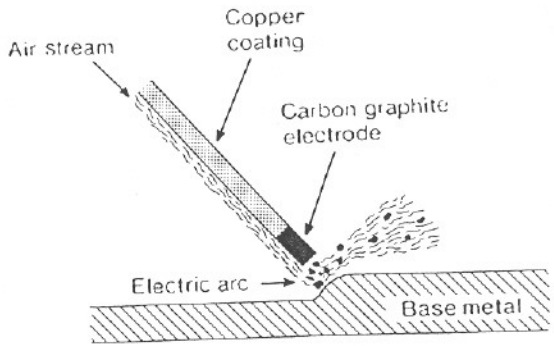

Figure 3. Arc-air gouging

2.5.2. Air contaminants

Arc-air gouging produces very high particle concentrations during brief periods. In an experiment designed to study exposure, a total particle concentration of 35 mg/m^3 was measured in the operator's breathing zone in 72 minutes (8). Nitrogen oxides, measured in the same experiment, were 2.8 mg/m^3 (2.1 ppm), 0.7 mg of which was NO_2; the carbon monoxide level was 9 mg/m^3 (8 ppm). Low concentrations of ozone were also recorded.

2.6. Air contaminants from breakdown of chlorinated hydrocarbons

Welding in air that contains organic solvents of the chlorinated hydrocarbon type can cause the formation of harmful gases by thermal or photochemical breakdown of the solvents and reactions with components in the air. Phosgene gas has attracted particular attention because of its ability to damage lung tissue (29, 30). Dahlberg et al (29) report that the photochemical pathway for formation of phosgene is more effective than thermal formation, and that the risk of phosgene formation from chlorinated hydrocarbons is greatest with MIG welding of aluminum, less for MIG welding of stainless and alloyed steel, and low for MAG welding of unalloyed steel with carbon dioxide as a shield gas. Dahlberg reports that under similar conditions perchloroethylene yields the highest phosgene concentrations, followed by trichloroethylene, which can also break down into dichloroacetyl chloride, whereas 1,1,1-trichloroethane yields small amounts of phosgene (29) and hydrogen chloride (29, 142).

2.7. Air monitoring

Concentrations of particles and gases are higher outside the welding helmets than inside them, largely due to the fact that the plume of welding fumes is carried away from the welder by thermal convection and gradually dispersed into the air, but also because the air exhaled by the welder dilutes the air inside the helmet. In one study it was found that the iron oxide concentration was 36 - 71% lower inside the helmet than outside it (58). If possible, therefore, air contaminants should be monitored inside the helmets.

2.7.1. Particles

In one standard method (7), samples are collected on membrane filters, usually of cellulose esters, with a pore size of 0.8 µm.

Total sampling time for a sample can range from an hour or so to an entire workday. Samples can be taken either at sampling stations or with personal samplers. In the latter case, the samples are taken inside the face-guard so that they will resemble the inhaled air as closely as possible. Face-guards or welding helmets are usually equipped with one or two metal casings made to hold a 37-mm filter, placed at mouth level on one or both sides of the head and angled so that they are not directly in the path of exhaled air. Sample hoses are attached to outlets in the casings (175).

When the helmet or face-guard is not being used for welding it is either raised or removed. The air sample is then assumed to correspond to background levels in the shop, which the welder is exposed to as long as he is there. When the welder leaves the workshop for an extended break, the sampler should be turned off and the time noted. The flow should be measured before and after the interruption.

The filter is weighed before and after sampling, and total dust is calculated. Most of the particles in welding dust are less than 1 µm in size, and virtually all the particles are respirable (78, 177).

With arc welding, the arc-time factor (the proportion of time the arc is ignited, a number between 0 and 1) is important in calculating the average contaminant level from the welding. The arc-time factor can either be estimated from time

studies or measured directly with an arc-time meter, a device clipped around the welding cable that registers the amount of time current is passing through the cable and the total work time (175).

The concentration of particles in the air during the time the arc is ignited can not be measured directly. A measure of exposure from welding when the arc is ignited, an immission index for the monitoring period, is obtained from the total contaminant level by subtracting the background level and dividing the result by the arc-time factor (175).

Several methods can be used for further analysis of the particle composition, such as atomic absorption for chromium and other elements. It can sometimes be practical to use multielement methods, particularly X-ray methods, either X-ray excitation or, at specially equipped laboratories, ion excitation (PIXE) (81). With PIXE, elements from 19 potassium upward can normally be analyzed with a detectability of 0.0001 mg/m^3 and an accuracy within 10% (175).

Chromium compounds containing hexavalent chromium can be determined with the diphenyl carbazide method (1) or the zephiramine method (53).

Chromium compounds with different solubilities in different media -- water, acid, alkaline -- are determined by leaching and subsequent atomic absorption analysis (17, 18, 101, 119). An alkaline solution has been reported to oxidize trivalent chromium (187). The use of chelating agents has also been described (170). Inter-laboratory calibration has been done for hexavalent and total chromium in welding fumes (178).

The need to further determine the sample composition varies. Benz(a)pyrene can be analyzed by fluorescence (166).

The fluoride on the filter can be determined by dissolving the filter in sulfuric acid, after which the fluorine is evaporated as hydrogen fluoride and collected in sodium hydroxide. The solution is then buffered and the fluoride concentration is measured with an ion-selective electrode (175).

When a particular welding method is used on a particular material, the particles in the air contain the material components in proportions that can be determined with some degree of accuracy. When these proportions are known, the concentrations of the individual components can be calculated from the total particle concentration (175), see also 3.5.11.

2.7.2. Gases

Air samples for analysis of nitrogen monoxide, nitrogen dioxide and carbon monoxide are obtained by collecting air for about 15 minutes in a foil-lined polyethene bag measuring about 60 x 60 cm (12 µm aluminum foil coated with 70 µm polyethene). Small samples for analysis are taken directly from the bag. When this method was tested, the concentrations of these gases remained unchanged for 40 minutes (175).

Analysis of nitrogen oxides is best done with chemiluminiscence, a method specific for these substances. Carbon monoxide can be analyzed with a direct-reading instrument containing an electrochemical cell. Nitrogen oxides and carbon monoxide can also be determined directly with analysis ampules, using air samples taken directly from the bag.

Ozone is determined with a direct-reading instrument based on the chemiluminiscence principle, a method specific for ozone. Before 1970, when

chemiluminiscence was introduced, calculations of ozone were probably not very accurate, as they would have been disturbed by nitrogen oxides. Judging from the extremely high ozone concentrations that were sometimes reported -- up to several hundred ppm in a few cases -- attempts to separate the ozone from the nitrogen oxides were probably not very successful either (177).

Organic substances, which can occur with welding of painted steel, are collected in a carbon tube using either a personal sampler or a motor-driven syringe, and analyzed by gas chromatography. The detection limit is estimated to be about 0.03 mg/m^3 for samples taken on an adsorbent, and 0.1 mg/m^3 for direct samples. Reactive substances such as aldehydes are suspected to break down when collected in a carbon tube, but can probably be detected by direct sampling (175).

3. Kinetics

3.1. Uptake

A 35-year-old welder who had worked at a shipyard for 11 years, welding low-alloy steel with coated electrodes, had 10 times more iron in his lungs than an unexposed subject (88).

When rats were exposed to 1500 mg/m^3 welding fumes from shielded metal-arc welding of low-alloy steel, the iron concentration in their lungs (freeze-dried tissue) rose from 500 to 1175 µg/g after 30 minutes of exposure, and from 500 to 7175 µg/g after four hours of exposure (71).

When rats were exposed (1 hour/day, 5 days/week) to welding fumes from metal-arc welding of low-alloy steel, the iron content in their lungs increased for the first 10 days and then remained constant (84). In a similar experiment, rats were exposed to welding fumes from metal-arc welding of stainless steel. Chromium in the lungs increased linearly during the first 20 days of exposure. Nickel increased also, but much more slowly (87).

3.2. Distribution

Rats exposed to 1500 mg/m^3 welding fumes from shielded metal-arc welding of low-alloy steel showed no increase of iron in blood, liver or kidneys after 4 hours of exposure (71).

When rats were exposed 1 hour/day, 5 days/week for 4 weeks to welding fumes (1.5 mg chromium/m^3) from metal-arc welding of stainless steel, the chromium content of their blood rose rapidly to about 0.5 µmol/l and then remained constant (87). Chromium in the liver showed no increase during the first week of exposure, and reached a maximum three weeks after exposure was terminated; in the kidneys it reached a maximum during the first week of exposure; and in the spleen it increased during exposure and continued to increase for two months after exposure was terminated (87).

In this experiment the rats were also exposed to 0.2 mg/m^3 nickel. Nickel concentrations did not increase in any organ except the lungs (87).

3.3. Elimination

Several metals, aluminum, barium, lead, chromium, manganese, nickel etc., are excreted to a greater or lesser extent via the kidneys (50). Fluoride is excreted mainly in urine (76) and carbon monoxide in exhaled air (126).

3.4. Biological half times

The aluminum content in the urine of welders who had been exposed to aluminum for less than one year dropped rather quickly, with a half time of 8 or 9 days. If the welders had been exposed for more than 10 years, however, excretion dropped only slowly over time: the biological half time was estimated to be half a year or more (154).

After rats were exposed to welding fumes from low-alloy steel for 4 hours, the iron content in the lungs had an initial rapid drop (half time about 24 hours) and then dropped more slowly (half time around 33 days) (71).

The iron content in the lungs of welders was determined by magnetopulmography. Clearance was estimated to be about 20% per year, indicating a half time of 3.5 years for iron in the lungs (86).

Welders who had been exposed to welding fumes from stainless steel excreted chromium from at least two compartments. During the first phase, the chromium in urine was halved in 4 to 41 hours (173, 181). Retired welders had 4 to 5 times as much chromium in urine as unexposed referents (181), indicating a later phase with a long half time.

3.5. Biological exposure indicators

3.5.1. Aluminum

Welders exposed to aluminum have more aluminum in blood and urine than unexposed referents (161). Urine content of aluminum after a workshift was observed to be dependent on both the day's exposure and the number of years the welder had worked with aluminum (160). The highest aluminum content measured in the blood of a welder, 2.5 µmol/l (161), is well below the serum content of 7.5 µmol/l proposed to prevent brain damage in dialysis patients (147).

Aluminum concentrations in blood and urine can be determined with atomic absorption spectrometry, using the graphite oven technique (105). Contamination from aluminum-containing dust is a major problem with aluminum analyses (179).

3.5.2. Barium

Electrodes with a coating containing barium fluoride or barium carbonate are used in some special cases. Welders who use these electrodes have a higher excretion of barium in urine after a workshift than do unexposed referents (32).

Barium can be determined by atomic absorption spectrometry.

3.5.3. Lead

Flame cutting and welding of steel coated with lead-based paint can result in very high lead exposure (171). Flame cutting other lead-contaminated materials is also risky (39). Lead in blood can easily exceed 1.5 - 2 µmol/l, the biological exposure limit proposed by WHO (185).

The most common analysis method for lead in blood is atomic absorption spectrometry.

3.5.4. Fluoride

Welders using fluoride-containing electrodes can have a fluoride concentration of 300 µmol/l in urine (188), well above the widely recommended limit of 210 - 260 µmol/l (72).

Fluoride can be determined with an ion-specific electrode.

3.5.5. Iron

Welding usually involves exposure to iron, mostly in the form of magnetite (Fe_3O_4). Iron inhaled into the lungs can be measured by magnetopulmography (82, 83, 107). A strong magnetic field is applied over the chest so that all the iron particles are oriented in the same direction, and the magnetic field induced over the chest is then measured. For welders, measurements of iron content have ranged from 4 to 2,000 mg (107). The method can probably be further developed (36, 85).

3.5.6. Cadmium

Flame cutting of cadmium-plated steel can involve exposure to high levels of cadmium oxide (14). The blood cadmium level can give a good indication of current exposure, while the urine level reflects body burden, provided there is no kidney damage (48).

The dominant analysis method for cadmium in blood and urine is atomic absorption spectrometry.

3.5.7. Carbon monoxide

Carbon monoxide is formed when carbon dioxide is used as a shield gas in MAG welding. Exposure can be assessed by measuring COHb in blood (34). COHb can sometimes exceed 5% (34), the limit recommended by WHO (184).

COHb can be determined by spectrophotometry or gas chromatography.

3.5.8. Chromium

Air concentrations of chromium or hexavalent chromium resulting from welding stainless steel with coated electrodes show a linear relationship to concentrations of chromium in the urine of welders after the workshift (158, 172, 181). Chromium in urine is dependent on excretion from at least two compartments, one with rapid excretion and another with slower excretion (173, 181), and can therefore provide only an approximate measure of exposure to chromium in welding fumes.

Chromium in urine can be determined by atomic absorption spectrometry.

3.5.9. Manganese

Welders exposed to manganese have somewhat higher manganese concentrations in blood and urine than unexposed referents. Manganese in a person's urine can vary considerably over time, probably because of the variation in intake via food.

Blood and urine concentrations of manganese can nonetheless be used to estimate group exposure (2).

Manganese can be determined by atomic absorption spectrometry.

3.5.10. Nickel

With welding of steel containing nickel, air concentrations of nickel show no correlation with concentrations in urine (189). Comparison of nickel concentrations before and after a workshift might give a better indication of nickel exposure during welding (140).

Nickel can be determined by atomic absorption spectrometry.

3.5.11. Total particle exposure

Some coated electrodes contain large amounts of calcium fluoride. When such electrodes are used, the fluoride in urine after a workshift can reflect the total particle exposure during the workshift (157).

Welders are usually exposed primarily to iron. Measuring the amount of iron in the lungs with a magnetopulmograph can therefore provide an estimate of total particle exposure; see 3.5.5.

4. General toxicology

Several gases formed during welding, such as nitrogen dioxide, ozone and in special cases phosgene, which can be formed if chlorinated hydrocarbons are present, can damage epithelial cells in the alveolae (type 1 pneumocytes) (59, 60) and thus cause pulmonary edema.

Welders who smoke cough up more macrophages than other smokers (123). Proteolytic enzymes from these cells are now being discussed as a possible factor in the development of emphysema in smokers (77). Alpha-1 antitrypsin counteracts the effect of the proteolytic enzymes, but this protein can be inactivated by e.g. nitrogen dioxide (118). Table 1 lists the metals that occur in welding fumes, along with their most important target organs.

Table 1. Metals in welding fumes and their critical organs.

Metal	Critical organs	Reference
Aluminum	Respiratory passages, CNS	42
Barium	Muscles	141
Cadmium	Respiratory passages, kidneys	48
Chromium (VI)	Respiratory passages (lung cancer)	104
Copper	Respiratory passages, metal fume fever	127
Iron	Respiratory passages	41
Lead	Central and peripheral nervous system	125
Manganese	Respiratory passages, CNS	55
Molybdenum	Respiratory passages	49
Nickel	Respiratory passages (cancer)	129
Niobium	Respiratory passages	40
Titanium	Respiratory passages	130
Tungsten	Respiratory passages	92
Zinc	Metal fume fever	128

5. Effects on organs

5.1. Respiratory organs

5.1.1. Metal fume fever

Metal fumes formed during welding can cause fever. Nearly 40% of welders over 30 years old have had metal fume fever. The most common cause was welding of galvanized steel (145).

5.1.2. Rhinitis

Chronic nasal inflammation (rhinitis) has been described in connection with welding of steel alloyed with manganese (182). Atrophic rhinitis has been associated to welding stainless steel with coated electrodes (80).

5.1.3. Reversible bronchial obstruction

Reversible bronchial obstruction can be either allergic or non-allergic.

Allergic bronchial obstruction has been described after exposure to welding fumes containing hexavalent chromium (28, 95).

A non-allergic bronchial obstruction can appear at ozone concentrations (46) like those measured around MIG welding of aluminum (175).

It has been suggested that a high single exposure to irritating gases can cause bronchial hyperreactivity that persists for a long time (20). Brooks et al describe a welder who had welded a tank used to hold acid. The welding produced a lot of fumes, and he began to cough. A few hours later he was not only still coughing but also had difficulty breathing. When he was examined four years afterward he suffered from bronchial obstruction that responded to bronchodilators, and a positive metacholine test indicated bronchial hyperreactivity (20).

A Danish study included 1,486 welders who had worked as welders more than 10 hours per week for at least 5 years, 1,019 welders who had been exposed for shorter periods, and 866 electricians. Asthmatic attacks in the form of wheezing and gasping had been experienced by 21% of the welders in the high-exposure group, 17% of those in the low-exposure group, and 11% of the referents (61). A higher frequency of bronchial hyperreactivity was also noted in the welders, and interpreted as a sign of easily irritated mucous membranes (121).

Welding of steel painted either with a zinc-epoxy primer or with a shop primer consisting of iron oxide and zinc tetraoxychromate as pigments, and polyvinyl butyral and phenol resin as binders, caused no bronchial obstruction in groups of welders (114, 135, 159).

There is a case report of a welder who worked with epoxy-painted steel on three different occasions during a year. On each occasion he developed a cough and had difficulty breathing, probable symptoms of bronchial obstruction (111). A similar case report from Finland describes a welder who developed severe bronchial obstruction after 5 minutes of welding iron painted with epoxy (94). Fever and bronchial obstruction have also been described after welding metals painted with epoxy-ester based chloropolymer lacquer (24).

Four welders who were probably exposed to isocyanate while working in the vicinity of polyurethane foam developed influenza-like symptoms and breathing difficulties (21).

5.1.4. Chronic bronchitis and emphysema

Chronic bronchitis is usually defined as productive cough daily for at least three months per year during the preceding two years (186). The symptoms are sometimes associated with bronchial obstruction. A greater prevalence of chronic bronchitis has been found in several studies of welders: see Table 2.

Table 2. Chronic bronchitis in groups of welders exposed mostly to fumes from welding low-alloy steel. The lower limit of the 95% confidence interval was greater than 1 in several studies (6, 10, 61, 135).

Type of work	Number exposed	Relative risk	Exposure	Reference
Railway tracks	149	3.3	Mould welding (median 5.3 mg/m^3)	
			overlaying (median 2.4 mg/m^3)	164
Workshop	39	3.1	–	10
Workshop	157	1.7	20 welders 1-2.5 mg/m^3	
			38 welders 3-4 mg/m^3	
			30 welders 6-9 mg/m^3	6
Workshop	91	1.0	average 3.9 mg/m^3	93
Workshop	346	1.1	3-26 mg/m^3	121
Workshop	305	1.2	3-317 mg/m^3	188
Shipyard	156	1.2	–	45
Shipyard	173	1.5	48-92 mg/m^3	
			in confined spaces	11
Shipyard	119	2.0	median 10 mg/m^3,	
			sampler on shoulder	135
Shipyard	1,486	2.7	–	61
Shipyard	135	1.1	–	116
Shipyard	526	2.8	–	27

Chronic bronchitis has been observed in welders working with low-alloy steel and also in welders working with stainless steel and aluminum (164). These studies are cross-sectional, which can mean that the relative risk is underestimated and any dose-response relationship weakened.

Reduction of FEV% (Forced Expiratory Volume over 1 second/vital capacity x 100) is an expression of bronchial obstruction. FEV% is not related to body height, and the average decrease in men is normally no more than 0.4% per year (152). Tables 3 and 4 summarize the studies that included over100 welders, most of whom worked mainly with low-alloy steel. The shipyard welders showed on average 4% lower FEV% than their referents. One English study also indicated a correlation between exposure and reduction of FEV% in smokers (27). For welders who did not work at shipyards, the average FEV% quotient was 1.0; see Table 4.

Table 3. Quotient of FEV% based on average results from studies covering more than 100 shipyard welders and referents. The average exposures of the welders ranged from 17 to 33 years.

Welders/referents	Smoking habits	Referents	Reference
0.94	Nonsmokers	Shipyard workers	74
0.87	Smokers		
1.00	Nonsmokers	Office workers	135
0.97	Smokers		
0.97	Nonsmokers	Shipyard workers	3
0.98	Smokers		
0.91	Nonsmokers	Dockworkers	116
1.01	Smokers		

When nonsmoking shipyard welders were compared with office workers (135) and electricians (110), they showed a higher closing volume and closing capacity and a steeper slope of the alveolar plateau. These findings indicate changes in the small peripheral airways. Changes, primarily in the small airways (FEF 25-75) but also in the larger ones (FEV_1), have been observed in shop welders exposed to welding fumes for 40 years or more but not exposed to asbestos (96).

Higher mortality due to chronic bronchitis was noted among welders and flame cutters in two American studies (12, 117). Higher mortality due to non-malignant respiratory diseases was also found among welders and flame cutters in a Swedish register study (112).

Table 4. FEV% quotient based on average results from studies that included more than100 non-shipyard welders and referents. The average exposures of the welders ranged from 12 to 21 years. One study (65) does not report exposure time.

Welders/Referents	Smoking habits	Studied groups	Reference
0.98	50% smokers 50% nonsmokers	Workshop welders vs. Non-welders	6
1.00	Nonsmokers	Rail welders	162
1.00	Smokers	Track layers	
1.01	Nonsmokers	Workshop welders	121
1.00	Smokers, <20 cigarettes/day	Blue-collar workers	
0.96	Smokers, >20 cigarettes/day		
1.11	Nonsmokers	Workshop welders	65
1.01	Smokers	Non-welders	
1.01	Nonsmokers	Workshop welders	188
1.03	Smokers	Metal workers (non-smoking referents had a notable decrease over time)	153
0.97	Nonsmokers	Workshop welders	96
0.98	Smokers	Men from Michigan	

5.1.5. Pulmonary edema

Pulmonary edema has been described in connection with both gas welding and flame cutting. It has been attributed to the high concentrations of nitrogen dioxide created by these processes (106, 131).

Inhalation of ozone formed during gas-shielded arc welding has also been reported as a cause of pulmonary edema (98). MIG welding of aluminum is the combination of material and method that generates the highest ozone concentrations.

High concentrations of cadmium oxide can be formed during flame cutting of cadmium-plated steel, and this exposure can cause pulmonary edema (14).

Welding in air that contains chlorinated hydrocarbons may form phosgene, which can cause pulmonary edema (5, 30, 31, 57, 163).

A welder developed a fatal case of pulmonary edema as a result of an accident at work. To identify the direct cause, an experiment was arranged to duplicate the welding conditions. MAG welding of oily, unalloyed steel in the presence of 1,1,1-trichloroethane resulted in 0.4 ppm phosgene when the work was done in a sampling booth. With open-air welding of the same basic material, however, the concentration of 1,1,1-trichloroethane was 260 ppm at the workbench, but there was no detectable phosgene in the breathing zone of the welder (detection limit 0.01 ppm). Measured concentrations of ozone, nitrogen oxides, sulfur dioxide (the oil on the workpiece contained sulfur), hydrogen chloride and chlorine were low (63, 148). There was thus no sure explanation for the lung damage.

5.1.6. Pneumonia

Some studies of welders have indicated increased mortality due to pneumonia (12, 115). This finding was not confirmed in a Swedish register study (152), but the study can be criticized for inadequate comparability between the welders and the referents.

5.1.7. Pulmonary fibrosis

Shadows in the lung X-rays of welders were first described by Doig and McLaughlin in 1936 (37), and interpreted as an indication of iron oxide deposits (siderosis) rather than pulmonary fibrosis. Ten years later the same authors described a welder whose lung X-ray changes had disappeared after exposure had stopped, and another welder in whom the changes had regressed when exposure was reduced (38).

Lung X-ray examinations were given to a group of 661 welders who worked in heavy industry in Scotland. The prevalence of small, round opacities was 5% after 20 years of welding and 20% after 35 to 40 years of exposure (9).

Several large cross-sectional studies of welders have produced no conclusive evidence of restrictive lung function changes as an expression of pulmonary fibrosis (6, 74, 121, 135, 164). Restrictive changes have been observed in welders, however. Peters et al found both restrictive and obstructive changes, in spite of normal X-rays, in welders who worked at shipyards (137). Pulmonary fibrosis and restrictive changes have been described in several case reports (22, 25, 51, 165).

Welders are exposed to several substances in addition to iron oxide. Welding has been associated with exposure to non-crystalline silicon dioxide (165), asbestos (37), and fluorides from the electrode coatings; metals such as aluminum

(69), cadmium, chromium, manganese and nickel from the electrode cores; and gases such as phosgene, nitrogen dioxide and ozone created by the welding process. Welders working around sand blasting can also be exposed to crystalline silicon dioxide, and they can be exposed to asbestos in shipyards (146, 149) and other heavy industries (9).

Combinations of these substances may cause the fibrosis sometimes seen in welders.

5.2. Kidneys

High concentrations of cadmium oxide can be formed during flame cutting of cadmium-plated steel, and long exposures can cause damage to kidney tubules (14). Welding stainless steel with coated electrodes has not been associated with changes in kidney function (109), while welding reinforcement steel with a special electrode caused higher urine concentrations of beta glucuronidase, an expression of disturbed tubular function. Welding in general, however, is not asssociated with chronic kidney disease (62).

5.3. Digestive system

Nausea and vomiting are sometimes seen in association with metal fume fever after exposure to zinc (144).

5.4. Nervous system

In one study, welders exposed to aluminum showed several symptoms of effects on the central nervous system when compared to other welders (156).

Welding or cutting steel coated with lead-based paint is associated with risk for damage to the central and peripheral nervous system (125).

After arc-air gouging of manganese-alloyed steel, two welders developed CNS symptoms that were an expression of high manganese exposure (183). In a group of welders exposed to 1 - 4 mg manganese/m^3 during MAG welding for an average of 16 years, there was a correlation between the number of years of exposure and an integrated reaction-time measure (150). Rail welders exposed to manganese also showed more CNS symptoms when compared to rail welders not exposed to manganese (156).

There is also some suspicion of a connection between welding and development of multiple sclerosis (44). No specific agent has been implicated, however.

5.5. Ears

Large particles formed during welding (welding beads) can penetrate the tympanum, causing pain and nystagmus. This acute phase can be followed by reduced hearing and chronic infections. Facial paresis has also been described (124, 180).

5.6. Blood and blood-forming organs

Welders showed an elevated count of white blood cells in peripheral blood (132). It has been observed in several studies that smokers have elevated leucocyte levels in peripheral blood, which may be an indication of an inflammatory condition in the respiratory passages (52, 138).

Anemia can appear in welders as well as in other occupational groups with exposure to substantial amounts of lead (125)

6. Immunotoxicity and allergies

Welding stainless steel with coated electrodes results in the formation of hexavalent chromium, which can cause reversible bronchial obstruction (95). This asthmatic reaction has been prevented with both sodium chromoglycate and cortisone (95).

Contact sensitization from exposure to hexavalent chromium in welding fumes is probably rare. Facial dermatitis, however, has been described after such exposure (47).

Urticaria has been described in connection with welding (43, 91). A welder developed itching and swelling (angioedema) of the face, lips and throat after exposure to zinc during work with galvanized steel (43), and another developed a fever and rash after welding steel sections containing polyurethane (89).

7. Mutagenicity and genotoxicity

7.1. Mutations

Welding of stainless steel creates particles that are mutagenic in bacterial tests. Particles from metal-arc welding cause more mutagenic activity than those from gas-shielded arc welding (MIG) (66, 113). Welding particles from low-alloy steel have also been noted to cause mutations (15).

7.2. Chromosome damage

Particles formed by metal-arc welding or gas-shielded arc welding of stainless steel caused increased frequencies of sister chromatid exchanges and chromosomal aberrations in cultures of hamster lung cells (102). Hamster ovarian cells showed more sister chromatid exchanges after the same exposures, and also after exposure to fumes from welding cast iron and low-alloy steel with coated electrodes. Particles from MIG welding of low-alloy steel, however, caused no increases (35).Welders doing shielded metal-arc welding of stainless steel had the same frequencies of sister chromatid exchanges and chromosome aberrations in peripheral lymphocytes as unexposed referents (75, 108). In other studies, welders working with both metal-arc welding and gas-shielded arc welding of stainless steel had higher frequencies of chromosome aberrations (99, 103) and impaired DNA repair (99).

8. Carcinogenicity

A 30% higher incidence of lung cancer among welders has been found in several studies (152, 168). Exposure to asbestos, nickel and hexavalent chromium may explain these results.

About 40% of the welders working in heavy industry in Scotland had been exposed to asbestos, primarily from mats and cloths used for heat insulation (9). Pleural plack and lung fibrosis, signs of asbestos exposure, occur among welders who work in shipyards (146, 149). Welders have also shown an increased incidence of mesotheliomas (13, 115, 152), a form of cancer that is strongly associated with exposure to asbestos.

Hexavalent chromium and nickel are released during the welding of stainless steel. Several studies have shown a connection between this kind of welding and the development of lung cancer (56, 97, 155). An increased number of tumors originating in the nose and nasal sinuses has also been observed in such welders (70).

Suspicions of a connection between welding and tumors in the larynx (133) and kidneys (117, 152) have also been expressed in recent years.

Several studies of welders give a relative risk of developing cancer in the bladder or lower urinary tract: reported values range from 0.6 to 2.8 (26, 73, 143, 151, 152).

Welding does not seem to be associated with the development of leukemia (169). Two studies, however, show a higher incidence of Hodgkins' lymphoma in welders (54, 136). None of these authors implicate any particular substance in welding fumes.

9. Reproduction toxicology

Our knowledge of reproductive disturbances among welders is very limited. In Finland, female welders have a somewhat higher frequency of miscarriages than other industrial workers, though the difference is not statistically significant. Wives of male welders had the same frequency of miscarriages as the wives of other industrial workers (67). Two Danish case-referent studies report a higher frequency of defective sperm in welders working with stainless steel. In one study (139) the odds ratio was 1.7 (95% confidence interval 1.0 - 2.8), and in the other (120) it was 2.34 (0.95 - 5.73). A cross-section study, however, revealed no difference in sperm quality between such welders and referents (79). About 25% of the welders in the second study worked with coated electrodes, and the differences in welding methods might explain this difference in results.

Wilms' tumor is a kidney tumor that occurs in children. Some studies suggest a connection between this form of tumor and the father's occupation as welder (23, 90, 134). Suspicions have not been directed toward any specific substance in welding fumes.

10. Exposure-response relationships

The higher prevalence of chronic bronchitis among welders is not clearly correlated to exposure: see Table 2. Exposure data is scarce, and it is difficult to estimate a typical exposure for any particular group of welders.

Bronchial obstruction in the form of reduced FEV% has been observed in some groups of welders. Welders in shipyards have shown a 4% average reduction of FEV% when compared with referents that did not do welding. Welders who did not work in shipyards, however, showed no reduction in FEV%. The difference in FEV% between shipyard welders and non-shipyard welders can probably be explained by differences in exposure. When total particle concentrations were monitored in a Danish shipyard (in the breathing zone in confined areas), 50% of the time-weighted average measurements exceeded 7 mg/m^3 (167), while with welding of railway rails (175) only 25 - 50% of the averages exceeded 5 mg/m^3. It is not clear whether the particle concentration as such is correlated to bronchial obstruction. Total particle concentrations may be regarded as an indicator of the concentrations of the various particles and gases formed by welding.

Reversible bronchial obstruction can occur after exposure to isocyanate, ozone or hexavalent chromium, and after welding steel painted with epoxy.

Welders can develop pulmonary edema after exposure to phosgene, cadmium oxide, nitrogen dioxide or ozone. The dose-response relationships for these substances can be found in the appropriate Criteria Documents.

The higher incidence of lung cancer observed in welders can probably be explained by exposure to asbestos, nickel and hexavalent chromium. These substances have also been discussed in earlier Criteria Documents.

11. Research needs

The occurrence of cancer in different groups of welders with adequately monitored exposures should be analyzed. Initiative for such studies has been taken by the International Agency for Research on Cancer (IARC) in Lyon.

Our understanding of reproductive disturbances among welders is extremely limited. This area should be given further study.

Exposure to aluminum and manganese during welding, and their effects on the central nervous system, should be studied more thoroughly.

12. Discussion and evaluation

The particles formed during welding are smaller than 1 µm, and therefore respirable. Exposure to the particles and gases formed by welding can cause chronic bronchitis, and at higher exposures also bronchial obstruction. With particle concentrations below 5 mg/m^3 there is probably little danger of bronchial obstruction.

Some gases that can be formed during welding, such as phosgene, nitrogen dioxide and ozone, can cause pulmonary edema. Welding should be done with

extreme caution if the air contains traces of chlorinated solvents, as these may form gases that cause pulmonary edema.

Lung cancer and asthma are the critical effects associated with welding in stainless steel.

With welding in steel painted with lead-based paint or alloyed with manganese, the critical organ is the central nervous system.

Welding of galvanized (zinc-plated) steel may cause metal fume fever.

13. Summary

B. Sjögren and U. Ulfvarson: Welding gases and fumes. Nordic Expert Group for Documentation of Occupational Exposure Limits. Arbete och Hälsa 1990:XX, pp 1-

Welders are exposed to several kinds of gases and particles. This document identifies the airborne substances associated with different welding techniques and different basic materials, and discusses the effects of exposure.

Many different welding operations are related to the development of chronic bronchitis among welders with long-term exposures. Bronchial obstruction has been observed among welders in shipyards.

Exposure to hexavalent chromium, isocyanate, ozone, and fumes from welding epoxy-painted steel may result in reversible bronchial obstruction.

Exposure to cadmium oxide, nitrogen dioxide, ozone and phosgene may cause pulmonary edema.

Exposure to asbestos, hexavalent chromium and nickel increases the risk for lung cancer.

Exposure to lead, manganese and possibly aluminum may affect the nervous system.

In English; 189 references.

Key words: gases, metals, occupational exposure limit, welding.

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Appendix I. Allowed or recommended maximum concentrations of welding fumes in workplace air.

Country	mg/m^3	Year	Reference
Denmark[1]	1.6-3.1	1988	2
France[2]	5	1988	6
Great Britain	5	1988	3
Netherlands	5	1986	4
Norway[3]	5	1984	1
U. S. A. (ACGIH)[4]	5	1988-89	5

1. Process- determined exposure limit
2. Total fumes
3. Not specified
4. TLV-TWA

References to Appendix 1

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SUMMARY

Beije B, Lundberg P (eds). Criteria documents from the Nordic Expert Group 1990. Arbete och Hälsa 1991:2, pp 1–317.

The Nordic Expert Group is a standing committee with the task to produce criteria documents on health effects of occupationally used chemicals. The documents are meant to be used by the regulatory authorities in the five Nordic countries as a scientific basis for the setting of national occupational exposure limits.

The volume consists of English translations of the cirteria documents which have been published in a Scandinavian language during 1990.

Key words: Criteria document, Dimethyldithiocarbamates, N-Nitroso compounds, Nordic Expert Group, Occupational exposure limits, Organic acid anhydrides, Styrene, Tiurames, Welding gases and fumes.

SAMMANFATTNING

Beije B, Lundberg P (eds). Kriteriedokument från Nordiska Expertgruppen 1990. Arbete och Hälsa 1991:2, pp 1–317.

Den Nordiska Expertgruppen är en arbetsgrupp med uppgift att producera kriteriedokument om hälsoeffekter av kemiska ämnen i arbetsmiljön. Dokumenten skall användas av tillsynsmyndigheterna i de fem nordiska länderna som ett vetenskapligt underlag vid fastställande av hygieniska gränsvärden.

Volymen omfattar en engelsk översättning av de kriteriedokument som har publicerats på ett skandinaviskt språk under 1990.

Nyckelord: Dimetylditionkarbamater, Hygieniskt gränsvärde, Kriteriedokument, N-Nitrosoföreningar, Nordiska Expertgruppen, Organiska syraanhydrider, Styren, Svetsgaser, Tiuramer

APPENDIX

Documents published in English by the Nordic Expert Group.

Acetonitrile	Arbete och Hälsa 1989:37, pp 149-174
Creosote	Arbete och Hälsa 1988:33, pp 7-51
Diacetone alcohol	Arbete och Hälsa 1989:37, pp 59-78
Hydroquinone	Arbete och Hälsa 1989:37, pp 79-114
Methyl bromide	Arbete och Hälsa 1987:40, pp 7-44
Methylene chloride	Arbete och Hälsa 1987:40, pp 74-120
Methyl formate	Arbete och Hälsa 1989:37, pp 175-202
Methyl isobutyl ketone	Arbete och Hälsa 1988:33, pp 53-76
n-Decane and n-Undecane	Arbete och Hälsa 1987:40, pp 45-73
Nitrilotriacetic acid (NTA) and its salts	Arbete och Hälsa 1989:37, pp 115-148
Nitroalkanes	Arbete och Hälsa 1988:33, pp 115-163
Paper dust	Arbete och Hälsa 1989:37, pp 203-246
Toluene	Arbete och Hälsa 1989:37, pp 7-58
Vinyl acetate	Arbete och Hälsa 1988:33, pp 77-113

Documents published by the Nordic Expert Group (NEG) in collaboration with the Dutch Expert Committee for Occupational Standards (DEC) or the National Institute for Occupational Safety and Health (NIOSH).

7/8-Carbon chain aliphatic monoketones (DEC & NEG)	Arbete och Hälsa 1990:2, pp 1-44
Propylen glycol ethers and their acetates (NEG & NIOSH)	Arbete och Hälsa 1990:32, pp 1-47
Ethyl acetate (NEG & DEC)	Arbete och Hälsa 1990:35, pp 1-36

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