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Scientific Basis for Swedish Occupational Standards XXXI

Swedish Criteria Group for Occupational Standards
Ed. Johan Montelius
Swedish Work Environment Authority
S-112 79 Stockholm, Sweden

Translation:
Frances Van Sant

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Preface

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

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

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

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

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

This publication is the 31th in the series, and contains consensus reports approved by the Criteria Group from July, 2009 through September, 2010. The consensus reports in this and previous publications in the series are listed in the Appendix (page 121).

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Johan Montelius
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The Criteria Group has the following membership (as of September, 2010)

Maria Albin		Dept. Environ. Occup. Medicine, University Hospital, Lund
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Jonas Brisman		Occup. and Environ. Medicine, Göteborg
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Ulla Stenius		Inst. Environmental Medicine, Karolinska Institutet
Marianne Walding	observer	Swedish Work Environment Authority
Håkan Westberg		Dept. Environ. Occup. Medicine, University Hospital, Örebro
Olof Vesterberg		

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[1] Drafted by Ilona Silins, Inst. Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.
[2] Drafted by Birgitta Lindell, Swedish Work Environment Authority, Sweden.
[3] Drafted by Hans Welinder, Division of Occupational and Environmental Medicine, Lund University, Sweden, and Johan Montelius, Swedish Work Environment Authority, Sweden.

Consensus report for asphalt fumes around road paving, with focus on bitumen fume

April 14, 2010

The asphalt uses receiving most attention in the scientific literature are paving and roofing. This document focuses on the health effects of asphalt fumes generated during road paving work.

The term “asphalt fumes” is necessarily imprecise, since the composition of asphalt fumes varies with the composition and treatment of the bitumen, one of its main components, and with the additives used. Further, asphalt based on coal tar produces fumes that are different from those produced by bitumen-based asphalt. The composition of the asphalt fumes is not mentioned in many studies, and was probably at least partly unknown. The results of epidemiological studies, for example, may have been affected by the earlier use of asphalt based on coal tar or by additives, which may or may not have been known of by the authors.

In American studies the term *asphalt fumes* is used (asphalt = bitumen in the USA), and the National Institute for Occupational Safety and Health (NIOSH, USA) has defined asphalt fumes as “the cloud of small particles created by condensation from the gaseous state after volatilization of asphalt” (50). The term *bitumen fume* is commonly used in European studies.

This document is concerned primarily with the cloud of small particles formed during condensation of the gas phase after bitumen in asphalt is heated during the process of road paving. Contributions from other substances are likely in many studies, however. The term “asphalt fumes” is generally used in this document, but in the descriptions of individual studies the terminology used by the authors has been followed as closely as possible.

The most recent literature search was made in PubMed in January of 2010. This report also draws on a DECOS document published in 2007 (20). The abbreviations used in the text are explained in Appendix 1 at the end of the document.

Physical and chemical data. Occurrence and use.

CAS No:	8052-42-4
Synonyms[1]:	bitumen, asphalt
Boiling point:	>400°C (at 101.3 kPa)
Melting point:	30 – 130°C
Combustion temperature:	>230°C
Solubility in water:	insoluble
Relative density:	1.0 – 1.18 kg/dm^3 (25°C)
(water = 1)	1.0 – 1.95 kg/dm^3 (15°C)
Flash point:	>400°C
Distribution coefficient:	>6
(log $P_{o/w}$)	

Asphalt consists mostly of crushed rock, with a small amount of binder in the form of bitumen (usually 5 – 7%) (54). Bitumen is a dark brown to black, non-volatile, adhesive and water-repellent substance. At room temperature it is extremely viscous or nearly solid, but softens on being heated. It is made in refineries by distillation of crude oil. The basic product of the distillation process is a bitumen that is heated to about 160°C for production and laying of asphalt on roads with low, medium and high traffic. All bitumens are complex mixtures of hydrocarbons with high molecular weights. A large proportion of these hydrocarbons are paraffins (alkanes) and naphthenes (cycloalkanes). There are also traces of metals: iron, nickel and vanadium. The exact composition depends on the type of crude oil used in production. Bitumen also contains polycyclic aromatic hydrocarbons (PAH), which vary with the type of bitumen (16, 54, 57). Several PAH (e.g. benzopyrene) are known to be both genotoxic and carcinogenic. Bitumen contains about 0.1 – 3 mg/kg benzopyrene (57). Bitumens are now produced to meet a range of different technical specifications, and production includes other bitumen products such as soft bitumen (bitumen mixed with softeners, often in the form of a heavy oil distillate) and bitumen emulsion (bitumen particles suspended in water containing surfactants such as amines/ammonia compounds) (54).

"Steam cracked bitumen" is an older term and refers to bitumen distilled in a vacuum. This type of bitumen is common in Sweden, and is used mostly as a binder in asphalt for road paving. "Oxidized bitumen" has been treated with air (partial blowing) and is used in Sweden mostly for roofing (20; personal communication, Anna Hedelin, Nynäs AB, 2009).

[1] "Asphalt tar" or "road tar" is a mixture of bitumen and tar. The mixture is no longer used. It is not the same as modern asphalt.

In road construction crushed rock and bitumen are heated and mixed at an asphalt plant and then applied to the road surface by a paving machine. The characteristics of the asphalt can be modified by changing the type and size of stone and type of bitumen. For some applications various additives are used, including adhesives (e.g. amines, 0.2 – 1.5% of the binder's weight), mineral additives such as slaked lime or cement (added in closed systems, 1 – 2% of total weight), polymer-modified binders (may contain e.g. styrene-butadiene-styrene chains, 2.6% polymers in bitumen), fibers of cellulose or mineral wool, release agents (e.g. diesel oil), as well as other substances such as recycled asphalt, granulated rubber from old vehicle tires, thickeners and coolants (e.g. waxes and natural asphalt). It is becoming more and more common to use a wide range of additives to modify and improve the functional characteristics of the asphalt (2, 54). It may be assumed that this affects the composition of the asphalt fumes, but there are few scientific studies in which the health effects of these additives have been examined.

Three types of asphalt surfacing are now used: hot mixed (>120°C), warm mixed (50 – 120°C), and cold mixed (about 50°C) (54). The asphalt used for road paving in Sweden (and elsewhere) is heated to 149 – 177°C, and on application the temperature ranges from 112 to 162°C. In Sweden, use of asphalt heated to lower temperatures has become more prevalent (a common type is mixed with soft bitumen and bitumen emulsion and heated to 80 – 120°C) (54). The application temperature of asphalt used in roofing is about 230°C (16). *Mastic asphalt* is a type of asphalt put down as a protective and insulating layer on e.g. bridges, parking garages and streets. Mastic asphalt is a mixture of fine sand, crushed stone, finely ground limestone and bitumen (12 – 17%). The application temperature is around 225°C, much higher than for conventional asphalt (29, 62).

Asphalt fumes are defined as the cloud of small particles formed by condensation of the gas phase after asphalt (=bitumen) has been heated (50). The components in the gas phase do not all condense at the same temperature, which means that workers are exposed to both asphalt fumes and gases. The composition of asphalt fumes cannot be described precisely, since it varies with factors such as the temperature of the asphalt, the production process and the ingredients in the asphalt mixture (63). The temperature of the asphalt affects both the amount of fumes formed and the content of PAH in the fumes. Asphalt fumes produced at high temperatures probably contain more PAH than fumes formed at lower temperatures (50). Coal tar was once widely used as an additive, which contributed to relatively high concentrations of PAH in the fumes. The concentration of benzopyrene, for example, is estimated to have been 100 times higher in fumes from asphalt containing coal tar (57).

Sixty million tons of bitumen and 700 million tons of asphalt are produced annually in industrialized countries (57). Most of it is used for paving and roofing (16). Sweden has an annual asphalt production of 6 to 7 million tons (39). About 0.85 million tons of bitumen were produced in Sweden in 2009, and about 0.5

million tons are used annually (personal communication, Matz Wiklund, Nynäs AB, 2010).

There are about 4000 asphalt mixing plants in western Europe, with 5 to 10 employees per plant. About 100,000 people work in road crews applying asphalt to the roads (16). An estimated 2800 Swedish workers are so employed (personal communication, Björn-Inge Björnberg, SEKO, 2010).

Road paving with asphalt involves several different jobs. A paver operator drives the paving machine, which spreads the asphalt on the road. A screedman follows the paving machine and evens out the edges and thickness of the asphalt. Rollers (driven by roller drivers) are then used to pack down the asphalt surface. Manual distribution of the asphalt with shovels and rakes is often involved as well (done by rakers) (31). Production, transport and application of the asphalt exposes the workers to asphalt fumes, mostly by inhalation but also via skin and digestive tract (16).

Levels in the work environment

There are several methods for measuring asphalt fumes and gas, but none of them has been shown to be specific, and it is hard to describe total exposure to asphalt fumes. Many studies have used total particle content (TPM or TP: Total Particulate Matter) and/or the benzene-soluble fraction of the total particle content (BSM or BSP: Benzene-Soluble Matter/Particles). The BSM method is used to measure the benzene-soluble particles that become airborne as a result of an industrial process, and the method is standard in the USA. Another method becoming more common is to measure the total organic content (TOM: Total Organic Matter = total hydrocarbons).

The asphalt fumes to which road pavers are exposed have been analyzed in several studies. In a study made by NIOSH, designed to develop and test new methods of defining asphalt fume exposure and to identify any health effects associated with exposure to asphalt fumes, data were collected from seven different road paving locations. The results showed that the concentration of asphalt fumes (measured with personal monitors) during a workday was generally below 1.0 mg/m^3 TPM and 0.3 mg/m^3 BSM (51).

In a study from 2007 (31) designed to define the physical and chemical characteristics of asphalt fumes and vapor from hot asphalt in road paving work, inhalation and skin samples were analyzed. In the samples, the PAH profile was dominated by substances with mol weights below 228 (relatively small PAH – benzopyrene, for example, has a mol weight of 252 g/mol). Substituted and heterocyclic PAH accounted for about 71% of the detectable mass concentration of PAH. The authors found that the particle phase from both the air samples and the skin samples was predominantly PAH with mol weights greater than 192. PAH concentrations in the air samples were higher in the gas phase but had lower mol weights than in the particle phase. Most of the particles in the gas phase were small (mass median aerodynamic diameter 1.02 μm). The measured asphalt fume

concentrations (TPM) for paving jobs were in the range 1.3 – 1.4 mg/m^3 for paver operators, 0.4 – 1.1 mg/m^3 for screedmen and 0.58 – 0.62 mg/m^3 for rakers.

The levels of total polycyclic aromatic compounds (PAC[2]) measured at a wavelength of 370 nm (which detects primarily small particles with 2 or 3 rings) were 197 – 198 μg/m^3 for paver operators, 52 – 206 μg/m^3 for screedmen and 51 – 55 μg/m^3 for rakers. PAC were also measured at 400 nm (which detects primarily larger PAH with 4 to 6 rings): levels were 35 – 39 μg/m^3 for paver operators, 9 – 40 μg/m^3 for screedmen, and 8.4 – 11 μg/m^3 for rakers. An earlier study reports a similar result for total PAC (51).

The largest epidemiological study of asphalt fume exposure and cancer is the retrospective European multi-center study made by the International Agency for Research on Cancer (IARC). One of the sub-studies contains semiquantitative estimates for bitumen fume exposure around road paving: 0.15 mg/m^3 (geometric mean, 95% Geometric Confidence Interval (GCI) 0.13 – 1.2) for paving work and 0.12 mg/m^3 (geometric mean, 95% GCI 0.07 – 0.20) around asphalt mixing. Estimated benzopyrene levels were around 2.0 ng/m^3 (geometric mean, 95% GCI 1.6 – 2.5) for paving and 2.4 ng/m^3 (geometric mean, 95% GCI 1.3 – 4.0) for mixing (12).

A study made in 2004 investigated PAC exposure of road pavers via skin contact and inhalation. The study also measured exposures for different paving jobs. Whole-day inhalation and skin samples were taken from 20 pavers on 3 workdays. The concentrations were found to be 4.1 μg/m^3 for inhalation and 89 ng/cm^2 for skin exposure (geometric means). Exposures to pyrene were 0.18 μg/m^3 for inhalation and 3.5 ng/cm^2 for skin contact. The concentrations of benzopyrene were also measured, but were below the detection limit (<0.01 μg/m^3 for air samples and <0.8 ng/cm^2 for skin samples). The paving crews had significantly higher exposure levels than the road workers who were not directly exposed to the hot asphalt (45).

McClean *et al.* also investigated exposures for different paving jobs. The average air concentration of PAH (measured as pyrene) was determined to be 0.6 μg/m^3 for paver operators, 0.5 μg/m^3 for screedmen, 0.2 μg/m^3 for rakers and 0.06 μg/m^3 for roller drivers (analysis of the total inhalation during an entire workday and sum of particle and gas phase). The highest skin exposures were measured for rakers (6.4 ng/cm^2) and screedmen (7.7 ng/cm^2); skin exposures were lower for paver operators (5.1 ng/cm^2) and roller drivers (below the detection limit) (samples for an entire workday and average for right and left wrists) (46).

Sweden is recycling more and more old asphalt. The old asphalt paving is crushed and used as filler or roadbed material. Asphalt can be recycled either hot, warm or cold. A problem with hot reprocessing is that older asphalt often

[2]The difference between PAH and PAC is that PAH has only carbon and hydrogen atoms (which may be substituted) in the aromatic benzene rings, whereas in PAC the benzene rings include other atoms as well (e.g. oxygen and nitrogen). The terms are sometimes used incorrectly (3).

contains coal tar, and the resulting fumes may contain high levels of PAH (19). Cold reprocessing is therefore used as often as possible, with asphalt temperatures around 80°C (personal communication, Björn Samuelson, Byggindustrierna, 2009). Several monitoring measurements have been made around asphalt reprocessing in Sweden, and the results have shown, for example, levels of 0.05 – 0.15 μg/m^3 for benzopyrene (40). Most measurements have been made around cold or warm processing, and in a few cases around hot mixtures with a temperature of 160°C. The PAH content of all the recycled material used in the study was analyzed in advance. The recycled material used in hot processing contained lower levels of PAH/kg dry substance than material used in cold or warm processing.

Uptake, biotransformation, excretion

Bitumen is a complex mixture of organic substances with high molecular weights, and also contains traces of metals. Each substance has its own pharmacokinetics, which probably changes on interaction with the other substances. The literature contains no information on uptake, biotransformation or excretion of asphalt fumes and bitumen, but there is information on some of its components, including PAH and long-chain aliphatic hydrocarbons (20). PAH are absorbed via both dermal and respiratory epithelium, and these are the primary paths of uptake. PAH are metabolized by the cytochrome P450 system, primarily to epoxides and various hydroxylated metabolites (64). Epoxides are often reactive metabolites that can bind to macromolecules such as DNA and proteins, where they may have toxic and mutagenic effects.

Uptake of asphalt components via skin and respiratory passages was investigated under controlled conditions in a study with volunteers. Ten men, non-smokers and previously unexposed, were exposed to bitumen B65 (20 mg/m^3, 2.5 mg/m^3 in the particle phase and 17.9 mg/m^3 in the gas phase). The production temperature was 200°C. The men stayed in an exposure chamber for 8 hours, with a 45-minute break after 4 hours. They wore only shorts during the exposure. Breathing masks were worn by 8 of them to prevent inhalation of the bitumen fume; the other two did not wear breathing masks. Exposure was measured as metabolites of PAH (pyrene, chrysene, phenanthrene) in 24-hour urine samples. Control samples were collected before the exposure. The total amounts of PAH metabolites after both skin and inhalation exposure were 370 ng/g creatinine for 1-hydroxypyrene, 690 ng/g creatinine for 6-hydroxychrysene and 85 ng/g creatinine for hydroxyphenanthrene (extrapolated from graph). The percentage levels of PAH metabolites after skin exposure alone were 58% for pyrene, 56% for chrysene, and 53% for phenanthrene (20), i.e. over half of the metabolites came from skin uptake.

An Italian study of paving workers measured air levels for 15 different PAH and skin exposure for 16 PAH (including phenanthrene, pyrene and benzopyrene), as well as urine levels of some PAH (pyrene and phenanthrene) and PAH metabolites (1-hydroxypyrene and 3-hydroxyphenanthrene) (24). There were 24 asphalt workers in the study (22 – 59 years old), wearing shorts and T-shirts. The asphalt

fumes came from asphalt containing 4 – 6% bitumen heated to 130 – 170°C. Three or four days into the workweek, skin exposure to PAH during a workshift (10 hours) was measured by applying polypropylene pads to various parts of the body (neck, shoulders, upper arms, wrists, groin, ankles). Air levels were measured with personal monitors for the first four hours of the workshift and urine samples were taken before and after the workshift, and also on Monday morning after at least two days away from work (base level). The total PAH deposition on the skin was calculated to be 86 µg (highest concentration on wrists): 25 µg for phenanthrene (highest on wrists), 7.4 µg for pyrene (highest on wrists) and 1.1 µg for benzopyrene (highest on the neck). The measured urine concentrations are shown in Table 1. Multiple linear regression analysis was used to test the association between 1-hydroxypyrene or 3-hydroxyphenanthrene in post-shift urine samples as dependent variables and air levels of pyrene or phenanthrene, skin deposition (wrists) of pyrene or phenanthrene, and base levels of 1-hydroxypyrene or 3-hydroxyphenanthrene as independent variables. The analysis showed that 42% of the variation in PAH metabolites in post-shift urine samples is explained by air exposure, skin deposition and base levels of metabolites. Skin deposition accounts for 12% (3-hydroxyphenanthrene) to 20% (1-hydroxypyrene) of the total variability. The authors concluded that the wrists are the best location for measuring skin deposition and that exposure via both skin and respiratory passages contributes to systemic exposure to PAH, and that the relative contribution depends on the substance (24).

Biological exposure monitoring

No biomarkers specific for asphalt fumes have been reported. However, there are several biomarkers that are used to assess exposure to asphalt fumes, such as excretion of hydroxylated PAH metabolites (e.g. 1-hydroxypyrene, a metabolite of pyrene), metabolites of thioether or glucaric acid in urine, analysis for adducts of

Table 1. Air levels of PAH and PAH metabolites and urine levels of PAH metabolites in the study by Fustinoni *et al*. (24).

Substance	Air levels, ng/m^3 (range)	Urine levels ng/l (range)		
		Base level	Pre-shift	Post-shift
PAH	565 (127-1165)			
Phenanthrene	33 (11-93)	17 (9-43)	18 (11-88)	34 (15-82)
Pyrene	32 (1.2-282)	4 (<4-7)	5 (<4-15)	5 (<4-11)
Benzopyrene	0.42 (0.13-7.8)			
3-OH-Phenanthrene		0.04 (<0.01-0.4)	0.09 (<0.01-1.7)	0.39 (<0.01-12)
1-OH-Pyrene		0.13 (<0.02-0.99)	0.22 (<0.02-3.7)	0.42 (<0.02-1.7)

DNA and proteins, and oxidative damage (not further described) in peripheral blood cells (9, 20). Pyrene is one of many PAH in asphalt fumes, and its metabolite 1-hydroxypyrene is often used to measure exposure to asphalt fumes. (It is also sometimes used as a biomarker for exposure to creosote or other PAH.) A study published in 2007 proposes a Clara cell protein (CC16) from blood as a biomarker for damage to pulmonary epithelium caused by occupational exposure to asphalt fumes (67).

McClean *et al.* monitored PAH exposure during a workweek for 20 pavers and 6 controls. Before work on Monday morning both groups had the same level of 1-hydroxypyrene in urine (0.8 μmol/mol creatinine, = 0.4 μg/g creatinine). The average level of 1-hydroxypyrene rose significantly in the pavers after each workshift, and after 4 workdays it was 3.5 times higher than on Monday morning. No increase was seen in controls. Different kinds of paving work were associated with different levels of 1-hydroxypyrene: screedmen > rakers > paver operators > roller drivers (46).

Toxic effects

Human data

Skin

There are several reports describing skin burns from direct contact with heated asphalt (not asphalt fumes) (20). Long-term skin exposure to asphalt fumes/bitumen fume can cause skin irritation, dermatitis, itching and rash (16, 60). The level at which these effects appear, however, is not clear, nor is it clear whether the symptoms are due to fumes only or to hot asphalt directly on the skin. Further, there is often skin exposure to other substances: coal tar, mineral fibers, formaldehyde, quartz dust, diesel exhaust etc. (20).

In one study, the expression of proteins associated with apoptosis (cell death) was examined in skin samples from 16 road pavers chronically exposed to bitumen fume (13 ± 6 years). A thinning of exposed epidermis and changes in protein expression (bax, bcl-2 and cytokeratin) may indicate elevated cell death induced by bitumen fume (42).

Respiratory system

Irritation of eyes, nose and throat has been reported by workers exposed to bitumen. A group of Norwegian researchers studied respiratory symptoms and lung function in 64 asphalt workers and a reference group of 195 construction workers. Symptoms in lower respiratory passages, allergies, medically diagnosed asthma and smoking habits were identified by questionnaire. The subjects whose spirometry results had an FEV_1/FVC ratio (forced expiratory volume for the first second/forced vital capacity) of <0.7 in combination with chronic cough, breathlessness and/or wheezing were given the diagnosis COPD (Chronic Obstructive Pulmonary Disease). Persons who reported medically diagnosed asthma were assumed to have asthma. The asphalt workers reported respiratory symptoms to a

greater degree than the reference group and had higher incidences of both asthma and COPD, see Table 2. The FEV_1/FVC ratio was 0.78 for the asphalt workers and 0.80 for the reference group ($p<0.01$). No information on exposure levels is given (58).

A group of 140 asphalt workers and a control group of 126 construction workers were tested with spirometry before and after the work season. The participants were also given a questionnaire. It was found that FEV_1 and FEF_{50} (forced mid-expiratory flow) were significantly lower in the asphalt workers (respectively 93% and 85% of expected normal values) than in the reference group (97% and 93% of expected normal values). Screedmen showed the greatest decline in lung function during the season compared with paver operators and roller drivers, see Table 3a. Although the paver operators, screedmen and roller drivers were exposed to significantly higher levels of PAH (1.3 – 1.8 $\mu g/m^3$) than the other asphalt workers (0.3 – 0.5 $\mu g/m^3$), the exposure levels were considered low to moderate (and were below the occupational exposure limit) (68). Total dust and oil mist were also analyzed, see Table 3b. There was no correlation between PAH in air and decline in lung function. The low number of observations in each subgroup, however, makes the interpretations uncertain.

Table 2. Workers with respiratory symptoms, COPD and asthma: asphalt workers compared to a reference group (58).

Symptom	Asphalt workers % (number of subjects)	Reference group % (number of subjects)	OR (95% CI)
Eye irritation	22 (14)	9 (18)	2.8 (1.2-5.9)
Chest tightness	22 (14)	9 (18)	2.8 (1.3-5.9)
Shortness of breath (climbing stairs)	11 (7)	3 (6)	4.1 (1.3-13.0)
Wheezing	29 (25)	21 (40)	2.6 (1.4-4.9)
Diagnosed asthma	14 (9)	2 (4)	7.9 (2.3-26.8)
COPD	19 (12)	8 (15)	2.8 (1.2-6.5)

Reference group = 195 construction workers (outdoor work).
OR = odds ratio (adjusted for smoking and age).
CI = confidence interval.

Table 3a. Changes in lung function of asphalt workers after the work season, according to job category (68).

Job category	Pre-season FVC	Change in FVC	Pre-season FEV_1	Change in FEV_1	Pre-season FEF_{50}	Change in FEF_{50}
Paver operator (n=16)	4.9 ± 0.5	0.05 ± 0.3	3.9 ± 0.5	0.04 ± 0.2	4.9 ± 1.9	0.13 ± 0.7
Screedman (n=42)	4.8 ± 0.8	-0.13 ± 0.4[a]	3.7 ± 0.6	-0.09 ± 0.3[a,b]	4.6 ± 1.5	-0.34 ± 1.3
Roller driver (n=12)	4.3 ± 0.7	0.05 ± 0.3	3.4 ± 0.4	-0.04 ± 0.1	4.4 ± 1.4	-0.28 ± 0.6
Asphalt stripper (n=6)	4.9 ± 1.4	0.21 ± 0.3	3.9 ± 1.5	-0.04 ± 0.2	4.9 ± 2.7	-0.87 ± 1.1
Asphalt mixing plant worker (n=30)	4.6 ± 0.8	-0.01 ± 0.3	3.5 ± 0.6	-0.02 ± 0.2	4.2 ± 1.6	-0.21 ± 0.8
Asphalt truck driver (n=18)	4.5 ± 0.8	-0.01 ± 0.3	3.5 ± 0.6	-0.04 ± 0.2	4.2 ± 1.5	0.11 ± 1.2

FVC = Forced Vital Capacity.
FEV_1 = Forced Expiratory Volume during the first second.
FEF_{50} = Forced Expiratory Flow at 50% of FVC.
[a] $p<0.05$
[b] $p<0.05$. Screedmen compared to other asphalt workers, adjusted for smoking.

Table 3b. Exposure levels according to job category, geometric means and geometric standard deviations (68).

Job category	Total dust (mg/m^3)	Total PAH ($\mu g/m^3$)	Oil mist (mg/m^3)
Paver operator (n=16)	0.3 ± 1.9	1.8 ± 1.9[b]	0.23 ± 3.4
Screedman (n=32)	0.3 ± 2.5	1.6 ± 2.2[b]	0.09 ± 2.3
Roller driver (n=8)	0.4 ± 2.7	1.3 ± 4.3[b]	Not reported
Asphalt stripper (n=9)	2.4 ± 1.5[a]	0.5 ± 1.8	0.19 ± 2.6
Asphalt mixing plant worker (n=9)	0.9 ± 1.8	0.5 ± 1.7	Not reported
Asphalt truck driver (n=10)	0.1 ± 2.4	0.3 ± 1.4	Not reported

[a] Asphalt strippers (removers of old asphalt) compared to other asphalt workers, $p<0.001$.
[b] Paver operators, screedmen and roller drivers compared to other asphalt workers, $p<0.001$.

A study by NIOSH (11) summarizes seven studies (all with the same protocol) of seven different paving jobs. Exposure to asphalt mixed with rubber from old tires and exposure to conventional asphalt were compared, including acute effects of the asphalt fumes. The studies lasted four days; rubber asphalt was used on two days and conventional asphalt on two days. There were 94 workers in the studies: 52 exposed and 42 unexposed. The subjects filled out a questionnaire on their health and were given frequent PEF (Peak Expiratory Flow) tests. Eye, nose and throat irritation were the most commonly reported acute symptoms (reported to be mild and temporary). Symptoms such as throat irritation were significantly higher in workers using conventional asphalt than in unexposed subjects (Odds Ratio 3.6, $p < 0.03$).

When the workers were exposed to the rubber asphalt, all the symptoms were significantly more severe ($p < 0.01$, OR for eye symptoms 4.0, nasal irritation 4.3, coughing 5.6, throat irritation 20.1). Four workers showed variation in lung function as measured in the periodic PEF tests, and for three of them it was judged to be work-related. The estimated average exposures for the asphalt workers ranged from 0.06 to 0.81 mg/m^3 TP and 0.02 to 0.44 mg/m^3 BSP while working with conventional asphalt, and 0.17 – 0.48 mg/m^3 TP and 0.02 – 0.25 mg/m^3 BSP while working with the rubber asphalt. On the days acute symptoms were reported on the questionnaire (eye, nose, throat irritation) by workers applying conventional asphalt, the concentrations of TP and BSP were significantly higher than on symptom-free days, but only TP was significantly higher on the days symptoms were reported by workers applying the rubber asphalt, see Table 4. Analysis of the asphalt fumes from both types of asphalt showed that PAC with 2 – 3 rings were more prevalent than those with 4 – 6 rings. The levels of organic sulfur-containing compounds and benzothiasol (a marker for added rubber) were higher for work with the asphalt containing rubber. There were also high levels of benzene (0.77 ppm) associated with the rubber asphalt work. The authors write that workers knew what type of asphalt they were working with each day and were uneasy about exposure from the rubber asphalt, which may have contributed to a bias in the symptom reports. The authors conclude that, although no clear dose-response relationship between asphalt fumes and acute symptoms could be identified, the reported symptoms suggest that there may be a causal relationship (11).

There is another study of 333 asphalt workers (79 using personal monitors) and a reference group of 247 maintenance workers without asphalt exposure. The workers with the exposure monitors were divided into subgroups of 5 or 6 workers representing different job categories (paver operator, raker and roller driver). The asphalt workers without personal monitors were subdivided in the same way. The average for one week of exposure to asphalt fumes was 0.36 mg/m^3 for the asphalt workers, and they had more symptoms than the unexposed group – abnormal fatigue, loss of appetite, eye and throat irritation (throat and pharynx) etc. The reported results were based on the symptom totals that showed a significantly higher frequency in the asphalt-exposed group than in the reference group.

Table 4. Average levels (mg/m^3, geometric means and ranges) of asphalt fumes on days with and without reported symptoms (eye, nose, throat irritation) (11).

Analysis method	Rubber asphalt			Conventional asphalt		
	No symptoms	Symptoms	p value	No symptoms	Symptoms	p value
TP	0.18 (0.01-0.78)	0.30 (0.04-1.38)	<0.01	0.13 (0.02-1.20)	0.23 (0.01-1.26)	0.02
BSP	0.08 (0.01-0.61)	0.13 (0.00-1.10)	0.26	0.05 (0.01-0.49)	0.16 (0.01-0.82)	<0.01

TP = Total Particulate Matter (mg/m^3).
BSP = Benzene-Soluble Particles (mg/m^3).

The symptom totals were 1.94 ± 0.22 for the asphalt workers with exposure monitors, 1.39 ± 0.10 for the other asphalt workers, and 0.75 ± 0.08 for the reference group (there was a significant difference between the latter two groups, $p < 0.001$). The workers laying asphalt in parking garages and tunnels reported significantly higher symptom totals than the other asphalt workers: 2.44 ± 0.54 vs. 1.25 ± 0.22 ($p < 0.05$). The symptom totals were not affected by weather, traffic density or specific job, but were significantly correlated to the temperature of the asphalt. Symptoms increased at asphalt temperatures of 145 – 155°C and also with an asphalt fume level above 0.4 mg/m^3: the symptom total for exposures below 0.4 mg/m^3 was 1.3 and the symptom total for exposures above 0.4 mg/m^3 was 3.0 ($p < 0.05$). The symptoms were not medically confirmed, but based only on the information reported in the questionnaires (52). A later analysis of the data yielded no correlation between symptom totals and exposure to the total amount of volatile substances/asphalt fumes (53). DECOS points out that this subsequent analysis was published only in an abstract, which contains no details on the models used (20).

Randem *et al.* investigated the correlation between asphalt work and mortality due to non-malignant diseases in a cohort of Norwegian asphalt workers. In the 803 deaths between 1970 and 1996, there was a not-significant elevation in mortality due to diseases involving the respiratory organs: Standardized Mortality Ratio (SMR) 1.3 (95% CI 0.97 – 1.6) (56).

Cardiovascular disease and inflammatory markers

Cardiovascular disease is nowadays often described as an inflammatory disease. In several meta-analyses a correlation has been observed between elevated levels of inflammatory markers in the blood (e.g. interleukin-6, C-reactive protein (CRP), fibrinogen) and elevated occurrence of coronary heart disease (17, 18). In one study, inflammatory markers were measured before and after the road-paving season. Interleukin-6 rose significantly during the season in nonsmoking asphalt pavers but there was not a significant increase of CRP (increased by 10%) or fibrinogen. The group of asphalt workers included paver operators, screedmen,

roller drivers and asphalt strippers (for exposure levels see Table 3b) (68). This result indicates a weak inflammatory reaction.

Correlation between PAH exposure and mortality due to ischemic heart disease was examined in a cohort study. In the cohort of 12,367 asphalt workers there were 418 cases of heart disease. Both cumulative exposure and estimated average exposure to benzopyrene were correlated to elevated mortality due to ischemic heart disease (dose-response). An average exposure of 273 ng benzopyrene/m^3 or higher corresponded to a relative risk of 1.64 (95% CI 1.1 – 2.4) (13). Simultaneous exposure to coal tar may have contributed to the high benzopyrene exposures: 273 ng/m^3 is very high compared to exposures reported by other studies taken up in this document.

In a study by Randem *et al.*, correlation between asphalt work and mortality due to non-malignant diseases was examined in a cohort of 8,610 Norwegian asphalt workers. No elevation in deaths due to cardiovascular disease was observed in the 803 deaths between 1970 and 1996 (56).

Mortality of asphalt workers was examined in a cohort of Danish workers. There were 1,320 men in the exposed group and 43,024 unexposed controls (men working in other jobs). The cohort was followed for 10 years (1970 – 1980), and during this time 113 asphalt workers and 3,811 unexposed subjects died. A not-significant elevation in deaths attributed to cardiovascular disease was seen: SMR 1.13 (95% CI 0.68 – 2.29) (28). No provision was made for smoking habits or other lifestyle factors, and the authors point out that the follow-up time was short.

Animal data

The asphalt or bitumen fumes used in most of the animal studies were generated in the laboratory, and in many cases are different in character from the asphalt fumes formed around road paving work.

A study with rabbits reports that direct application of "residues from a vacuum distillation of the residuum from atmospheric distillation of crude oil" caused slight irritation of skin and eyes (20). Dermatitis and local effects on skin were observed after both short- and long-term exposure to the bitumen condensate. Sores and small abscesses were seen after long-term exposure (20). Rabbits were given skin applications of "bitumen vacuum residuum distillation products" three times a week for four weeks. Lower food consumption was reported at 1000 mg/kg body weight, and minimal to moderate dermatitis and keratosis (increased growth of skin keratin) at 1000 – 2000 mg/kg b.w. (20).

Effects on respiratory passages

Rats that inhaled 10 – 58 mg/m^3 asphalt fumes (from asphalt heated to 170°C) for 5 days showed no indications of acute pulmonary effects. Examined markers included neutrophil infiltration, lactate dehydrogenase activity, reactive oxygen species (ROS) and production of proinflammatory markers such as Tumor Necrosis Factor Alpha (TNFα) and interleukin-1. However, with increasing total dose of asphalt fumes there was increasing activity of the metabolizing protein

CYP1A1 in bronchiolar epithelium (Clara cells) and a simultaneous decline of CYP2B1 activity (44). Rats exposed to the asphalt fume condensate by intratracheal instillation (0.1, 0.5 or 2.0 mg/day for 1 – 3 days) also showed no indications of acute pulmonary effects when the above-mentioned markers were examined (43).

Toxic effects of inhalation exposure to bitumen fume were studied in Wistar rats in order to determine concentrations and maximum tolerable dose for a future cancer study. The bitumen fume was generated to resemble exposure of road pavers in Germany. Sixteen rats per group were exposed to 4, 20 or 107 mg/m^3 bitumen fume 6 hours/day, 5 days/week for 14 weeks. None of the rats died from the exposure. The exposure to 107 mg/m^3 bitumen fume resulted in significantly lower body weights in the males, and also caused statistically significant exposure-related histopathological changes (hyalinosis, basal cell hyperplasia, mucous cell hyperplasia and inflammatory cell infiltration) in nasal cavity and sinuses. (CICAD points out, however, that p values are not given in the industry report from Fraunhofer summarized in Reference 16.)

Five days of exposure to 16 mg/m^3 bitumen fume caused irritation in the nasal cavities of rats (65). Mice that inhaled a bitumen-water aerosol for 16.5 to 21 months showed indications of pneumonitis, emphysema and bronchitis. The concentrations of bitumen fume/aerosol are not reported (20).

Effects on the immune system

A study published in 2008 (1) examined immunotoxic effects of asphalt fumes and asphalt fume condensate (generated at 150°C) on mice. The authors reported a dose-related trend ($p < 0.01$) with statistically significant suppression of the specific immunoglobulin M (IgM) response after systemic exposure (intraperitoneal injections) of the asphalt fume condensate (0.625 – 5 mg/kg b.w.). Intraperitoneal injection of the particle phase (5 mg/kg) and inhalation of asphalt fumes (35 mg/m^3) and asphalt fume vapor (11 mg/m^3) caused significant reductions in the specific reaction to erythrocytes from sheep (SRBC). Four days of skin exposure to asphalt fume condensate caused significant reductions in reaction to the total (at 50 mg/kg) and specific (at 250 mg/kg) IgM response after injection of SRBC. Immunosuppression was analyzed with the IgM plaque-forming cell response assay. The immunologic reactions to intravenous injection of SRBC with and without previous asphalt exposure were compared. The results showed that systemic exposure, as well as dermal and inhalation exposure, had immunosuppressive effects on mice.

Genotoxicity

In vitro

The effects and genotoxic potency of bitumen fume have been examined in several *in vitro* studies. Some of the studies have used bitumen fume produced in the laboratory at high temperatures (>200°C) (20). A number of *in vitro* studies

(some using Ames tests) have shown little or no mutagenic effects from bitumen fume produced at relevant temperatures (5). A recently published Finnish *in vitro* study with human bronchial epithelial cells (BEAS 2 cells) showed that asphalt fumes collected around road paving work or generated in the laboratory from mastic asphalt at about 150°C increased the number of micronuclei (MN), but there was no increase of micronuclei when the cells were exposed to asphalt fumes from conventional asphalt (41).

Animal data

The genotoxicity of bitumen fume was examined by exposing Big Blue© mice[3] to asphalt fumes (generated at 170°C, 100 mg/m^3 TPM, benzopyrene concentration 198 ng/m^3) via inhalation for 5 days. No difference in genotoxicity (mutations and adducts) was seen in the lungs when exposed animals and controls were compared four weeks after the end of exposure (48). A similar study in which Big Blue© rats were exposed by inhalation resulted in significant elevations of 1-hydroxypyrene in urine and DNA adducts in lung tissue, and weak (not significant) changes in mutation spectra in the target cells in the lungs of the exposed animals (8). Other *in vivo* studies showing genotoxicity (adducts and mutations) and changes in gene expression in pulmonary tissue have also used high concentrations (25 – 198 mg/m^3 TPM) of roadwork- or laboratory-generated bitumen fume (5, 20, 25, 27).

DNA damage in the form of DNA fragmentation was examined in alveolar macrophages and lung tissue from rats that had been exposed to asphalt fumes (25 or 38 mg/m^3 generated at 170°C) 6 hours/day for 5 days. The exposure resulted in DNA damage to both lung tissue and alveolar macrophages (determined by Comet assay). A single six-hour exposure to 59 mg/m^3 asphalt fumes also resulted in significantly higher levels of DNA damage (compared to controls inhaling clean air). There was a dose-response relationship to cumulative amount of asphalt fumes (mg-h/m^3). No increase of micronuclei in bone marrow erythrocytes could be detected after exposure to 58 mg/m^3 6 hours/day for 5 days (70).

The occurrence of DNA adducts was studied in lung tissue from 48 mice after inhalation exposure to asphalt fumes (generated at 180°C) 4 hours/day for 10 days. Levels of PAH-DNA adducts in the exposed mice were significantly higher than in controls breathing clean air. The exposure concentrations were in the range 152 – 198 mg/m^3 ("total exposure") (69).

The genotoxic effect of dermal exposure to bitumen fume was studied by applying a bitumen fume condensate to the skin of rats twice two days apart. Blood and tissue samples were taken for analysis of DNA adducts, and urine was tested for 1-hydroxypyrene. The condensate was absorbed very rapidly by the skin and resulted in adducts in skin, lungs and lymphocytes, but not in liver and kidneys. The adduct pattern was quite different from that caused by coal tar

[3] Big Blue© mice and rats are transgenic animals containing a vector that makes it easier to study mutations caused by exposure. Mutation frequencies (quantitative) and specific mutations (qualitative) can be detected in several different organs.

condensate (positive controls), probably because the bitumen contained greater amounts of heterocyclic PAH (especially those containing sulfur) than the coal tar. There was no correlation between the occurrence of adducts and the level of 1-hydroxypyrene in urine. The adduct pattern was also quite different for different organs, probably due to differences in organ-specific metabolism (26). The PAH concentrations (4 – 6 rings) in the bitumen condensates were 86 μg/g at 160°C and 94 μg/g at 200°C.

Human data

DNA strand breaks were examined in peripheral mononuclear blood cells from 34 asphalt workers (7 roofers, 18 road pavers, 9 bitumen painters). Blood samples were taken on Mondays and Fridays. For the roofers, it was found that the number of DNA strand breaks at the end of the workweek was significantly higher than in a control group (22). No information on exposure levels is given in this article.

Urine and blood samples from 28 asphalt workers and 28 controls were analyzed in a study published in 1998. The urine samples from the asphalt workers contained higher levels of 1-hydroxypyrene than those from the controls: 0.78 ± 0.46 vs. 0.52 ± 0.44 μmol/mol creatinine. Sister chromatid exchanges (SCE) and micronuclei (MN) were quantified in the blood samples. The asphalt workers had significantly higher levels of SCE per cell than controls: 5.13 ± 0.64 vs. 2.25 ± 0.42, $p < 0.05$. The frequency of MN in peripheral lymphocytes was also higher in the asphalt workers: 4.71 ± 0.67 vs. 1.79 ± 0.32 in controls, $p < 0.0001$. No information on air levels of PAH is provided (10).

SCE and MN frequencies were compared in a group of 28 Swedish road pavers and 30 unexposed controls, all nonsmokers. The average concentration of PAH that the asphalt workers had been exposed to was calculated to be 2.3 (range 0.2 – 23.8) $\mu g/m^3$. There was no significant difference between exposed workers and controls with regard to frequencies of micronuclei or sister chromatid exchanges. The level of 1-hydroxypyrene in the urine of the road pavers after 2 workshifts was the same as the pre-shift level (0.96 μmol/l). However, this level was about 30% higher than the level in controls (37). In this study 1-hydroxypyrene was not corrected for the amount of creatinine in urine.

MN in blood cells and exposed urothelial cells from 12 road pavers and 18 controls (hospital workers) were examined in an Australian study. The number of MN in urothelial cells was higher in the road pavers than in controls (12 ± 0.65 vs. 6.9 ± 0.18 MN per 1000 cells and 8.7 ± 0.46 vs. 5.2 ± 0.11 MN cells per 1000 cells). The lymphocytes of the road pavers also had higher levels of micronuclei than controls (16 ± 0.63 vs. 9.2 ± 0.29 MN per 1000 cells and 11 ± 0.24 vs. 5.9 ± 0.13 MN cells per 1000 cells). The differences between the road pavers and controls were significant ($p < 0.01$). PAH metabolites in urine were not analyzed, though smoking was taken into consideration (49).

In a study from 2006, no elevation in SCE levels was seen when a group of 19 asphalt workers were compared with a control group. Oxidative DNA damage was also analyzed using a formamido-pyrimidine-glycosylase (Fpg)- modified Comet

assay, and was found in 37% of the exposed group (0% in controls). In the exposed group 40% ± 12% of cells were found to have DNA fragmentation (comet assay) vs. 11% ± 4.5% in controls (p = 0.000, Anova test). The concentration of total PAH (mostly with 2 – 3 rings) was 2.8 (range 0.43 – 16) $\mu g/m^3$ for the exposed workers (15).

Correlation between exposure to asphalt fumes and the occurrence of DNA adducts in asphalt workers in Boston has been described in two works by McClean *et al.* (45, 47). In the DNA adduct study, 49 road pavers and 36 controls were followed for a year. Blood samples were taken from each person in the spring, summer, fall and winter. DNA adducts in mononuclear leukocytes were analyzed using ^{32}P postlabeling. During the work season (spring, summer and fall) the DNA adduct level in most of the road pavers rose from $3/10^{10}$ nucleotides on the first workday of the week to $46/10^{10}$ nucleotides after the fifth workday. The DNA adduct levels were highest for both exposed subjects and controls during the winter, and overall the controls had higher levels of DNA adducts. The exposure-related increase of DNA adducts during the workweeks with confirmed PAH exposure, however, suggests that road paving work leads to DNA damage.

In a study from 2009, exposure to asphalt fumes and SCE and MN were studied in 26 Turkish asphalt workers and 24 "matched" men with office jobs. The asphalt workers had significantly higher urine levels of 1-hydroxypyrene after a workshift than the controls. The study also reports higher levels of genotoxic markers in lymphocytes from asphalt workers than in those from controls (7.2 ± 1.6 vs. 5.5 ± 1.1 SCE per cell; 2.0 ± 0.21 vs. 1.5 ± 0.14 MN per 1000 binuclear cells). The frequencies of SCE and MN were higher after two weeks of paving work, but whether the increases were significant is not stated. According to the authors, the elevated levels of SCE and MN reflect mostly chronic exposure. No information on exposure levels for asphalt fume/PAH is given (38).

There has been some uncertainty about the genotoxic observations reported both *in vitro* and *in vivo*. *In vitro* partly because of different sources (condensate, solutions, extract) for the bitumen/asphalt fumes, and partly because the fumes used in the various studies were generated at different, and sometimes high (>230°C), temperatures. In studies of occupationally exposed workers, the results have been affected by differences in exposure situation, use of protective equipment, and treatment of confounding factors (including smoking and other simultaneous exposures). In 2007 the Dutch group DECOS concluded that PAH can penetrate the skin and form DNA adducts, and that the correlation between bitumen/asphalt fume exposure and genotoxicity is not clear (20). Several studies have been published since.

In summary, it is known from animal experiments that inhalation of high concentrations of bitumen fume generated at 170 – 180°C causes DNA damage in lung tissue of rats and mice (69, 70), and that dermal exposure to asphalt/bitumen fume condensate (generated at 160 or 200°C) causes DNA damage in the skin and peripheral tissues of rats (26). Further, studies of road pavers exposed to bitumen fume have documented elevated levels of DNA fragmentation, DNA adducts,

SCE and MN, as well as 1-hydroxypyrene in urine (10, 15, 38, 47, 49). Levels of MN, an established marker for cancer risk, were elevated for work with bitumen in three of the four studies published since 1998 (see Table 5).

Table 5. Observed effects on genetic material from occupational exposure to bitumen fume.

Material	Exposure/exposure marker		Effect	Result	Ref.
	PAH in air ($\mu g/m^3$)	1-hydroxypyrene in urine (µmol/mol creatinine)			
Peripheral blood, mononuclear blood cells, 27 bitumen-exposed (road pavers and mastic asphalt spreaders).			Single strand breaks (alkaline elution assay).	Negative	22
Peripheral blood, lymphocytes, 28 bitumen-exposed (road pavers), 28 controls (university and hospital employees).		0.78 ± 0.46 (after a week of occupational exposure) 0.52 ± 0.44 (controls, daytime)	Sister chromatid exchanges. Micronuclei.	Positive Positive	10
Peripheral blood, lymphocytes, 28 bitumen-exposed (road pavers), 30 controls (construction workers).	2.3 (geometric mean, range 0.2-24)	0.96 (0.04-3.8)* pre-shift 0.96 (0.23-4.0)* after 2 exposed workshifts 0.60 (0.14-2.2)* controls; afternoon samples	Sister chromatid exchanges. Micronuclei.	Negative Negative	37
Peripheral blood, lymphocytes, 19 bitumen-exposed (road pavers), 22 controls (office workers).	2.8 (mean, range 0.43-16)	0.52 (pre-shift) 1.5 (after 1 shift) 0.95 (controls, pre-shift)	Sister chromatid exchanges. DNA fragmentation (comet assay).	Negative Positive	15
Peripheral blood, lymphocytes, and cells from bladder, 12 bitumen-exposed (road pavers), 18 controls (hospital workers).			Micronuclei.	Positive	49
Peripheral blood, mononuclear blood cells, 49 bitumen-exposed (road pavers), 36 controls (millers).		0.8 ± 0.8 (pre-shift, n=20) 1.9 ± 1.9 (after 1 shift, n=20) 0.8 ± 0.6 (controls, pre-shift, n=6) 0.8 ± 0.8 (controls, after 1 shift, n=6)	DNA adducts.	Positive	45, 47
Peripheral blood, lymphocytes, 26 bitumen-exposed (road pavers), 24 controls (office workers).		0.18 ± 0.07 (pre-shift) 0.39 ± 0.21 (after 2 weeks of occupational exposure) 0.16 ± 0.008 (controls, pre-shift)	Sister chromatid exchanges. Micronuclei.	Positive Positive	38

* geometric mean, µmol/l (range).

Carcinogenicity

Human data

A major European multicenter study made by the IARC (55) has shown that the elevated risk of lung cancer previously observed among asphalt workers was very probably due to tobacco smoking, and possibly also to exposure to coal tar. Other epidemiological studies have reported elevated risk of bladder cancer among road pavers (14, 28, 57), and also stomach cancer (29, 30, 36, 57, 66), but the correlations to bitumen exposure are uncertain.

Some older epidemiological studies that examined correlations between asphalt fume exposure and cancer studied roofers or mastic asphalt workers, whose exposures differ from that typical for road pavers. In many cases it was impossible to exclude other simultaneous exposures that affected the result. A common confounding factor with asphalt work is simultaneous exposure to coal tar. To investigate the carcinogenic characteristics of asphalt fumes, the IARC gathered a very large retrospective cohort that included workers in the asphalt industry in seven European countries (including Sweden). The primary purpose was to determine whether an elevated risk of lung cancer could be correlated to exposure to bitumen fume. The cohort comprised 29,820 workers exposed to bitumen (road pavers, asphalt mixers and roofers) and 32,245 construction workers not exposed to bitumen. The cohort was followed from 1953 to 2000. The total mortality for the bitumen workers was lower than for the population in general. A small but statistically significant elevation in lung cancer cases was seen for the bitumen workers (SMR 1.17, 95% CI 1.04 – 1.30); for the other construction workers the SMR was the same as for the general population (SMR 1.0l, 95% CI 0.89 – 1.15). The relative risk of lung cancer for the bitumen workers compared to the other construction workers was 1.09 (95% CI 0.89 – 1.34). The results for individual countries varied somewhat. Mortality due to lung cancer was correlated to average exposure to bitumen fume but not to cumulative exposure or length of exposure. The study also revealed that road pavers had a relative risk of 1.34 (95% CI 0.93 – 1.94) for cancers of the ”head and neck”[4] (see Table 6). The SMR for these cancers was 1.37 (95% CI 0.98 – 1.88), and the SMR for lung cancer was 1.15 (95% CI 0.93 – 1.40). No adjustments were made for smoking, and the authors write that occupational exposure to other substances may have affected the results. The IARC's retrospective multi-cohort studies (6, 7) include some of the individual studies cited in the present document. These are Bergdahl and Järvholm 2003 (4), which is included in Randem *et al.* 2004 (59), which in turn is included in Boffetta *et al.* 2003 (6, 7).

[4] “Head and neck cancer” includes cancers of the mouth, nose, sinuses, salivary glands, throat and lymph nodes in the neck.

Table 6. Relative risk (RR) for some causes of death: road pavers compared to construction workers (6).

Cause of death	Relative risk (95% CI)
All causes	0.97 (0.90-1.0)
All cancers	0.96 (0.84-1.1)
Head and neck cancers	1.34 (0.93-1.9)
Lung cancer	0.99 (0.77-1.3)

A detailed sensitivity analysis for various assumptions used in the exposure estimates (including use of coal tar) has been published (21). According to the authors, the sensitivity to various assumptions regarding exposure estimates is relatively low.

Correlation between cancer and exposure to asphalt fumes was studied in Swedish construction workers (4). Cancer morbidity and mortality among asphalt workers were compared with a group of construction workers not exposed to asphalt fumes and to the general population. The study also traces the reduction in the use of coal tar in asphalt in Sweden since the middle of the 1970s. The authors estimate that PAH levels around roadwork in the 1980s and 1990s were in the range 1 – 2 $\mu g/m^3$ and that the level of bitumen fume was around 1 mg/m^3. The total numbers of cancer cases and cancer deaths were lower than predicted. Of 32 lung cancer cases, 24 were smokers at the first examination (the asphalt workers were followed from 1971 to 1995). Their relative risk of lung cancer compared to other construction workers, adjusted for smoking habits, was 1.03 (95% CI 0.70 – 1.45). Nor was there an elevation in risk for lung cancer among workers who had begun work with asphalt in 1954 or earlier (despite the use of coal tar in asphalt mixtures used before the 1970s), or for other types of cancer. This study is included in Randem *et al.* 2004 (59).

Cancer incidence after employment in the asphalt industry was investigated in a Nordic study. The study covered 22,362 asphalt workers in Denmark, Finland, Norway and Sweden. The national follow-up periods varied from 1953 – 1971 to 1994 – 1999. The asphalt workers were compared with the general population. During the follow-up periods 842 road pavers developed cancer: Standardized Incidence Ratio (SIR) 0.90 (95% CI 0.84 – 0.96). There were 50 cases of stomach cancer, SIR 1.29 (95% CI 0.96 – 1.70), 164 cases of lung cancer, SIR 1.26 (95% CI 1.08 – 1.47), 45 cases of non-melanoma skin cancer, SIR 0.52 (95% CI 0.38 – 0.70), and 62 cases of bladder cancer, SIR 0.94 (95% CI 0.72 – 1.20). The risk of bladder cancer increased with duration of employment: the relative risk (RR) was 1.85 (95% CI 0.90 – 3.78) with more than 30 years since initial employment (59). There was no control for smoking habits or other possible simultaneous exposures. Another limitation was that for the Norwegian part of the cohort the observation time had begun as early as 1953, although there were indications that until 1970 there was a selective attrition which, as the authors later pointed out, led to an underestimate of the cancer risk (57).

The correlation between occupational exposure to PAH while paving roads and risk for bladder cancer was investigated in a cohort study. The study included 7,298 workers whose only job had been paving roads and who were employed between 1913 and 1999 at various companies in Denmark, Norway, Finland and Israel. The authors estimated benzopyrene exposure (PAH) from historical changes in paving technology and exposure levels. There were 48 cases of bladder cancer among the road pavers; of these 39 had been exposed to asphalt fumes for at least 15 years prior to their diagnosis. No correlation between cumulative exposure to PAH and bladder cancer incidence was identified, but there was a correlation between average exposure level and bladder cancer, which was strengthened when a 15-year latency period was applied (RR 1.5 for 99 – <139 ng benzopyrene/m^3, 95% CI 0.54 – 4.4; RR 2.7 for 139 – <204 ng benzopyrene/m^3, 95% CI 1.0 – 7.3; RR 1.9 for >204 ng benzopyrene/m^3, 95% CI 0.66 – 5.5; p for trend 0.15) (14). The authors made no provision for smoking or other possible exposures.

Correlation between occupational exposures and risk for stomach (but not gastric cardia) cancer was calculated in a Swedish cohort study. The study cohort included 256,357 construction workers, all men, who had been employed in the Swedish construction industry between 1971 and 1993. The workers included in the cohort had previously, during medical checkups, answered questions on job history and smoking habits. There were 948 cases of stomach cancer that occurred during the 1971 – 2002 follow-up period. Of these, 14 has been exposed to asphalt fumes. High occupational exposure to asphalt fumes yielded an incidence rate ratio (IRR) of 0.9 (95% CI 0.5 – 1.5). Combined exposure to fumes (including asphalt fumes, diesel exhaust and metal fume) yielded an IRR for stomach cancer of 1.2 (95% CI 1.1 – 1.4) and included 262 exposed subjects (66).

Another cohort study of essentially the same population (260,052 male construction workers, followed from 1971 to 2000) investigated the correlation between occupational exposures and cancers of the esophagus and gastric cardia. Occupational exposure was classified by occupational hygienists, and information on smoking habits and body mass index (BMI) was obtained from physicals: 4,766 persons were classified as having high exposure to asphalt fumes. No increase in risk of esophageal cancer was seen, but there was an increase for cancer of the gastric cardia based on 6 cases (IRR 2.3, 95% CI 1.0 – 5.3, p 0.04) (36).

Cancer mortality in the asphalt industry was examined in a Danish cohort. The exposed group consisted of 1,320 men employed in the asphalt industry. The unexposed controls were 43,024 men working in other jobs. The follow-up time was 10 years (1970 – 1980), and a total of 113 asphalt workers and 3,811 unexposed subjects died during this time. The most common forms of cancer for the asphalt workers were cancer of the respiratory passages, SMR 1.4 (95% CI 0.82 – 2.3) and bladder cancer, SMR 3.0 (95% CI 0.98 – 7.0) (28). The exposure category was broad and included all types of asphalt work. Smoking habits and other

lifestyle factors were not considered. The authors note that the follow-up time was short.

All cases of bladder cancer in an area in Canada were included in a case-control study in which 835 persons with diagnosed bladder cancer were sent a questionnaire. The questions covered employment history, smoking and eating habits etc. A group of 781 matched controls also received the questionnaire. There was a significantly elevated risk of bladder cancer after work with asphalt/tar 8 to 28 years previously (OR 3.11, p 0.019). The job category "work with asphalt/tar" is broad, and the results are based solely on the subjects' own reports of exposure. The authors themselves point out that the responses from the study participants may not have been representative, since only 67% of the cases and 53% of the controls returned the completed questionnaires (61). Further, it is probable that these workers were also exposed to coal tar.

In two epidemiological studies (29, 30), Hansen investigated the correlation between severe effects on health and exposure to asphalt fumes. A Danish cohort of 679 mastic asphalt workers was followed from 1959 to 1986. During this time there were 169 deaths – a higher mortality rate than that of Danish men in general during the same time period, Standardized Mortality Ratio (SMR) 1.6 (95% CI 1.4 – 1,9), and cancer mortality was higher, SMR 2.3 (95% CI 1.7 – 2.9). There was an elevation of lung cancer among the mastic asphalt workers, SMR 2.9 (95% CI 1.9 – 4.3), as well as higher mortality due to liver cirrhosis, SMR 4.7 (95% CI 1.9 – 9.6). Among subjects over the age of 40 there were elevated incidences of cancers of the mouth, Standardized Morbidity Ratio 11 (2 cases, 95% CI 1.4 – 40), esophagus, SMR 7.0 (3 cases, 95% CI 1.4 – 20), colon, SMR 2.2 (7 cases, 95% CI 1.3 – 6.6) and lungs, SMR 3.4 (27 cases, 95% CI 2.3 – 5.0). Mortality due to bronchitis, emphysema and asthma was also elevated for mastic asphalt workers, SMR 2.1 (95% CI 0.95 – 3.9). No control for smoking, alcohol use or other lifestyle factors was made in this study. Mastic asphalt is heated to a higher temperature (around 230°C) than asphalt used in conventional paving, and contains more bitumen. Unlike conventional paving asphalt, mastic asphalt previously did not contain added coal tar as a binder (except for a brief period during World War II). In the late 1970s the Danish authorities measured extremely high levels of asphalt fumes around mastic asphalt work, especially flooring: 0.5 – 260 mg/m^3 (average 42 mg/m^3), but also around paving: 4.3 and 3.4 mg/m^3. The average concentration of PAH was 0.2 mg/m^3, and the average value for benzopyrene was 5.8 µg/m^3 (29, 30).

A case-control study (55) was made to study the weak correlation between bitumen exposure and lung cancer risk that had been observed in the earlier European cohort study described above (6, 7). Exposure estimates were made for bitumen fume, PAH from bitumen (inhalation exposure) and bitumen condensate (skin exposure), as well as for organic vapor, asbestos, quartz, diesel exhaust, smoking and coal tar. The estimates were based on earlier information from the companies and on personal interviews. In all 433 cases (male workers below 75 years of age, from Denmark, Finland, France, Germany, The Netherlands, Norway

and Israel, who had died of or been diagnosed with lung cancer between 1980 and 2002 – 2005) and 1,253 matched controls were included in the analysis. The odds ratio for lung cancer after bitumen fume exposure (at any time) was 1.1 (95% CI 0.84 – 1.5). There was no correlation between lung cancer risk and number of years of exposure, cumulative exposure or average exposure. Similar results were seen for exposures to PAH and organic vapors. Exposure to bitumen condensate (at any time) yielded an odds ratio of 1.2 (95% CI 0.88 – 1.6) for lung cancer, but there was no correlation to number of years of exposure, cumulative exposure or average exposure. However, it was found that cumulative exposure to coal tar correlated to elevated lung cancer risk. The odds ratio was adjusted for country, age and cigarette pack-years. According to the authors, two conclusions could be drawn from the study: that a large portion of the elevated mortality for lung cancer relative to the general population that had been observed in the earlier cohort study was probably due to high tobacco consumption among these workers and possibly also to coal tar exposure, and that other chemicals seemed to have no contributory effect. The authors state in summary that there is no consistent evidence of a correlation between lung cancer risk and bitumen exposure via either skin uptake or inhalation (55).

Animal data

Lung cancer

A two-year cancer study with rats was published in 2007. The rats, 50 in each dose group, were exposed to 6.8, 34.4 or 172 mg/m^3 (total hydrocarbons) bitumen fume. The asphalt fumes were regenerated in the laboratory from bitumen fume condensate collected above vats of hot bitumen stored at an asphalt plant. Analysis of individual PAH showed that the benzopyene level in 6.8 mg/m^3 bitumen fume was below the detection limit. Benzopyrene levels in bitumen fume at 34.4 and 172 mg/m^3 were 5 and 30 ng/m^3 respectively. Both hematological and histopathological analyses were made. Mortality was the same in all groups, but there was a significant reduction of average body weight (both males and females) at the two higher dose levels. Several non-neoplastic changes were significantly more common in exposed animals, especially in the higher dose groups. These included dose-related degenerative, inflammatory and proliferative changes in nasal cavity and lungs. The authors write that these may be potentially pre-neoplastic changes, but they found no indication of progressivity. There was no elevation in tumor incidence when treated rats were compared with controls. A single male in the highest dose group had a poorly differentiated adenocarcinoma in the nasal cavity. The authors concluded that bitumen fume was not carcinogenic to rats (23) (see Table 7).

Skin cancer

In most of the animal studies made to investigate correlation between skin cancer and exposure to asphalt/bitumen fumes, high temperatures (usually 200° – 300°C) were used to generate the condensate. A couple of studies report elevated occur-

rence of skin tumors in exposed rats. Since laboratory-generated bitumen/asphalt fume is different in character from the asphalt fumes around road paving work, it is difficult to interpret these results. The bitumen/asphalt fume condensates came from several different sources and were dissolved in different solvents, factors which may also contribute to the difficulty in interpreting the sometimes contradictory results of the animal studies (20).

Summary of earlier assessments

The IARC has placed bitumen in Group 3 ("not classifiable as to its carcinogenicity to humans") (32, 33). Coal tar is placed in Group 1 ("carcinogenic to humans") (34, 35).

Extracts of steam-cracked and oxidized bitumen (i.e. not fume from these products) have been classified by the IARC as "possibly carcinogenic to humans" (Group 2B). However, this classification is based on results of older studies in which steam-cracked/vacuum-distilled or oxidized bitumen was painted on skin, sometimes in undiluted form and sometimes dissolved in benzene or toluene. Most of these studies lack information on concentrations and/or control groups (32, 33).

Table 7. Non-neoplastic changes observed in the respiratory passages of Wistar rats exposed to asphalt fumes for 2 years (23).

Location	Males (controls, 6.8, 34.4, 172 mg/m^3 total hydrocarbons); 50 animals per dose group.	Females (controls, 6.8, 34.4, 172 mg/m^3 total hydrocarbons), 50 animals per dose group.
Nose/sinuses		
Olfactory epithelium: basal cell hyperplasia	0/1/1/20***	0/0/3/27***
Respiratory epithelium: hyperplasia	0/3/3/13***	0/0/2/20***
Goblet cell hyperplasia	1/11**/25***/46***	7/10/37***/47***
Olfactory epithelium: eosinophil cytoplasmatic inclusion	1/13***/16***/31***	12/11/27***/38***
Respiratory epithelium: eosinophil cytoplasmatic inclusion	2/5/7/22***	7/3/21**/24***
Mucosa: mononuclear/inflammatory cell infiltration	2/8/18***/27***	11/5/22*/34***
Respiratory epithelium: erosion	0/0/2/6*	0/0/0/1
Lungs		
Bronchiolar-alveolar hyperplasia, bronchiolar type	4/1/22***/46***	6/7/21**/44***
Alveolar histiocytosis	32/31/47***/50***	39/34/44/50***
Cholesterol granuloma cleft	0/0/2/6*	0/0/2/6*
Mononuclear/inflammatory cell infiltration	0/3/8**/3	0/0/6*/1
Lung-associated lymph nodes		
Macrophage accumulation (histiocytosis)	1/1/0/12**	1/2/5/26***

* $p<0.05$, ** $p<0.01$, *** $p<0.001$, significant difference from controls.

Both DECOS (20) and CICAD (16) consider that more studies are needed to determine whether bitumen fume is carcinogenic. DECOS regards bitumen as a suspected human carcinogen that has been insufficiently investigated.

Effects on reproduction

No reports of reproduction effects were found in the literature.

Dose-effect/dose-response relationships

The most important dose-effect/dose-response relationships are summarized in Table 8. Many studies lack information on air levels for exposure to asphalt fumes, and it is therefore difficult to establish a dose-effect correlation for some effects (such as lung function effects and human genotoxicity). It should be borne in mind that in many studies – especially field studies – the asphalt fumes are not fully described and the additives used are not known. It is therefore difficult to say what substance or substances in the fumes causes the observed effects and to what extent the individual studies are applicable to Swedish conditions. Levels of many PAH (e.g. benzopyrene, other genotoxic PAH) in the fumes from recycled asphalt used in road paving may be considerably higher than in conventional asphalt.

Respiratory passages

Symptoms of irritation of eyes, nose and throat, as well as coughing, have been reported with exposure to asphalt fumes (bitumen fume). These symptoms have been reported at 0.23 mg/m^3 TP and 0.16 mg/m^3 BSP for work with conventional asphalt. Addition of ground rubber to the asphalt increased the irritative effects at about the same exposure levels. On the days symptoms were reported the concentration of bitumen fume was nearly twice as high (0.30 mg/m^3 TP) as on symptom-free days (0.18 mg/m^3 TP) (11). Obstructive lung disease (COPD) and effects on lung function have been reported among workers exposed to asphalt fumes, but the studies are few and the evidence weak (58, 68).

Heart, inflammation, effects on immune defense

Mice exposed to asphalt fumes (35 mg/m^3) responded with decline in immune response. Similarly, intraperitoneal injections of asphalt fume condensate (0.625 – 5 mg/kg b.w.) yielded dose-dependent reductions in immune response (immuno-suppression) (1). In one epidemiologial study it was observed that elevated exposure to benzopyrene yielded an elevated risk of death due to ischemic heart disease (with both cumulative exposure and average exposure) (13). The asphalt workers had higher levels of interleukin-6 after the work season, which may indicate a weak inflammatory reaction. The geometric means for exposure to total dust ranged from 0.3 to 2.4 mg/m^3 (68).

Genotoxicity

In animal studies, DNA damage has been detected at concentrations in the range 38 – 59 mg/m^3 (total exposure) and 152 – 198 mg/m^3 (4 hours/day for 10 days) (27, 69, 70). In studies of human subjects, elevated levels of DNA strand breaks, DNA adducts and micronuclei have been observed in blood cells from road pavers exposed to asphalt fumes (10, 15, 38, 47, 49). Exposure levels in these studies are not known. Two studies showed that SCE levels were unaffected in workers exposed to asphalt fumes at PAH concentrations of 2.3 and 2.8 μg/m^3 (15, 37).

Cancer

Significant increases in hyperplasia (cell proliferation) were observed in nasal cavities and sinuses of rats after exposure to 6.8, 34, 107 and 172 mg/m^3 (total hydrocarbons). At 172 mg/m^3 one rat had a poorly differentiated adenocarcinoma in the nasal cavity (23). Cancer incidence and mortality after exposure to asphalt fumes have been investigated in several epidemiological studies. Some of these report elevated risks for bladder and stomach cancer (14, 28, 29, 30, 36), but the connection to bitumen is not certain. Most of these epidemiological studies did not factor in smoking habits. Further, in most of these studies broad groups of asphalt/bitumen workers were included, not just road pavers. There are several older studies showing elevated lung cancer risk after asphalt fume exposure, but none of them were adjusted for smoking habits (6, 7). Asphalt fumes (and bitumen fume) contain several PAH/PAC known to be carcinogenic and genotoxic, but a large European multi-center study made by the IARC in 2009, in which results were adjusted for smoking habits, showed no elevation in lung cancer risk after exposure to bitumen not containing coal tar (55).

Conclusions

There are no data on which to base a critical effect of exposure to asphalt fumes during road paving work. Irritation of eyes, nose and throat has been observed with exposure to 0.23 mg/m^3 (measured as total particles) and 0.16 mg/m^3 (measured as benzene-soluble fraction), but additives may have contributed to the effect.

Field studies of asphalt workers have demonstrated that asphalt fumes are genotoxic. No exposure levels are given in these studies, but they have been made at paving jobs using modern road-paving methods. It is possible that additives contributed to the effect. Genotoxicity has also been shown in animal studies, although with exposures to asphalt fumes generated at higher temperatures than are used in road paving.

Road paving work with asphalt (bitumen) may be carcinogenic. In those epidemiological studies where correlations have been found, however, the role of bitumen fumes is unclear.

Studies with volunteers suggest that with exposure to asphalt fumes skin uptake of polyaromatic hydrocarbons can be quite high.

Table 8. Dose-effect/dose-response relationships for exposure to asphalt fumes.

Concentration (mg/m^3)	Exposure time	Effects	Ref.
Human studies			
0.23 (TP) 0.16 (BSP)	2 workdays, conventional asphalt	Irritation of eyes, nose, throat. (52 road pavers)	11
0.30 (TP) 0.13 (BSP)	2 workdays, rubber asphalt	Irritation of eyes, nose, throat. (52 road pavers)	11
0.36 (asphalt fumes)	5 days	Irritation of eyes, nose, throat; fatigue, loss of appetite. (333 road maintenance workers)	52
0.0028 (PAH)	3 days	Oxidative DNA damage, DNA fragmentation (comet assay) in lymphocytes. (19 road pavers)	15
Animal studies			
6.8 (total hydrocarbons)	2 years	Non-neoplastic histological changes in nose and sinuses (rats).	23
16 (asphalt fumes)	5 days	Nasal irritation. (rats)	65
34 (total hydrocarbons)	2 years	Reduced body weight, non-neoplastic histological changes in nose and sinuses. (rats)	23
35 (asphalt fumes)	1 or 2 weeks	Effects on immune defense. (mice)	1
38 (asphalt fumes)	6 hours/day, 5 days	DNA damage in alveolar macrophages (comet assay). (rats)	70
50 (TPM)	5 days	DNA damage in lungs. (rats)	27
53 (asphalt fumes) 59 (asphalt fumes)	1 hour 6 hours	Increase of CYP1A1 activity. (rats) Reduction of CYP2B1 activity. (rats)	44
59 (asphalt fumes)	6 hours	DNA damage in alveolar macrophages (comet assay). (rats)	70
107 (total hydrocarbons)	6 hours/day, 5 days/week, 14 weeks	Reduced body weight, histopathological changes in nose and sinuses. (rats)	16
172 (total hydrocarbons)	2 years	Reduced body weight, non-neoplastic histological changes in nose and sinuses; one nasal carcinoma. (1/50 rats)	23
152-198 (TPM)	4 hours/day, 10 days	DNA adducts in lungs. (48 mice)	69
0.625-5*	Daily i.p. injection, 1 week	Effect on immunosuppression (dose-response). (mice)	1
1000*	Dermal application, 3 times/week, 4 weeks	Reduced feed consumption. (rabbits)	20
1000-2000*	Dermal application, 3 times/week, 4 weeks	Dermatitis, keratosis. (rabbits)	20

TP/TPM = Total Particulate Matter.
BSP = Benzene-Soluble Particles (benzene-soluble fraction of the TPM).
PAH = Polycyclic Aromatic Hydrocarbons.
* Asphalt (bitumen) fume condensate (mg/kg).

Potential conflicts of interest

Bengt Järvholm (member of the Criteria Group) reported that he had worked on a project that was funded partially by the Development Fund of the Swedish Construction Industry and that resulted in some published articles cited in this consensus report.

No other potential conflicts of interest have been reported.

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Appendix 1

Abbreviations

BSM/BSP	Benzene-soluble matter/particles, benzene-soluble fraction of total particle content
CI	Confidence interval
CICAD	Concise International Chemical Assessment Documents
COPD	Chronic obstructive pulmonary disease
CYP1A1	Cytochrome p4501A1
CYP2B1	Cytochrome p4502B1
DECOS	Dutch Expert Committee on Occupational Standards
FEF_{50}	Forced expiratory flow at 50% of FVC
FEV_1	Forced expiratory volume during the first second
FVC	Forced vital capacity
IARC	International Agency for Research on Cancer
IgM	Immunoglobulin M
IRR	Incidence rate ratio
MN	Micronuclei
NIOSH	National Institute for Occupational Safety and Health (USA)
OR	Odds ratio
PAC	Polycyclic aromatic compounds
PAH	Polycyclic aromatic hydrocarbons
PEF	Peak expiratory flow
RR	Relative risk
SCE	Sister chromatid exchange
SIR	Standardized incidence ratio
SMR	Standardized mortality ratio or Standardized morbidity ratio
SRBC	Sheep red blood cells
TPM, TP	Total particulate matter
TNF	Tumor necrosis factor
TOM	Total organic matter (total hydrocarbons)

Consensus Report for Formaldehyde

June 9, 2010

This report is based mostly on a criteria document from DECOS/NEG published in 2003 (32) and an IARC monograph published in 2006 (58). Further information was obtained from data searches of PubMed in September of 2009 and April of 2010. The Criteria Group published previous consensus reports for formaldehyde in 1981 (122) and 1983 (123).

Chemical and physical data. Uses

CAS No:	50-00-0
Synonyms:	methanal, oxomethane, oxymethylene, methylene oxide, methyl aldehyde
Formula:	CH_2O
Mol weight:	30.03
Melting point:	- 92°C
Boiling point:	- 20°C
Vapor pressure:	0.2 kPa (20°C)
Conversion factors:	1 ppm = 1.23 mg/m^3 1 mg/m^3 = 0.81 ppm (20°C, 101.3 kPa)
Other data:	Formalin: Aqueous solution of formaldehyde (sometimes with stabilizer such as methanol). Concentrated formalin usually contains 30 to 50% formaldehyde. Paraformaldehyde: Solid, low-molecular polymer of formaldehyde containing 90 to 99% formaldehyde. 1,3,5-Trioxane: Solid, cyclic trimer of formaldehyde.

Formaldehyde at room temperature is a colorless gas with a pungent odor (123). Most people can identify the odor at 1 ppm, and the odor threshold has been reported to be between 0.05 and 0.18 ppm. The substance is extremely reactive and is easily polymerized. It is also flammable, and can form explosive mixtures with air. Formaldehyde mixes with water and acetone and dissolves in ethanol. It occurs in aqueous solutions as hydrate. Formaldehyde can react with hydrogen chloride and other inorganic chlorides to form the carcinogenic substance 1,1'-dichlorodimethylether (11, 32, 58, 123).

Formaldehyde is a component of normal human metabolism and occurs naturally in fruits and other foods. The substance is also formed e.g. in decomposition or incineration of organic materials (11, 58). The primary industrial use of

formaldehyde is for production of resins from phenol, urea, melamine and polyacetal. The three first-named types of resin are used as adhesives/binders in paper and wood products, in production of plastics and in surface treatments for textiles, etc., whereas polyacetal resins are used mostly in production of plastics (58). Formaldehyde occurs in manufacture of various types of plywood, particle board, MDF board (medium-density fiberboard) etc. (100). Formaldehyde is formed by acid-cured varnishes during the curing process, although the varnishes themselves contain very little formaldehyde (100). Formaldehyde or formaldehyde-releasing substances are often used as preservatives in products such as cutting fluids and water-based paints. Formaldehyde is also used in pathology and cytology laboratories. Formaldehyde or substances that emit formaldehyde (formaldehyde emitters) are widely used as preservatives in cosmetics, hygiene products and foodstuffs (11, 58, 100; 2010-04-26 http://www.skane.se/templates/page.aspx?id=123138 [in Swedish]). Because of formaldehyde's allergenic properties, the EU cosmetics directive specifies that cosmetics and hygiene products may contain no more than 0.2% (74). Formaldehyde is classified as a contact allergen (R 43) with a specific classification limit of 0.2% (67).

The Swedish Work Environment Authority, in projects in 2004 and 2006, mapped formaldehyde exposure in various business sectors (100). Air levels of formaldehyde measured around application of acid-cured varnish were in the range 0.08 – 0.5 ppm (0.1 – 0.6 mg/m^3) in about half of 35 whole-day measurements; 3 values were >0.5 ppm (>0.6 mg/m^3), and 0.7 ppm (0.9 mg/m^3) was the highest measured level. Spray painting with acid-cured varnish and mixing varnish were reported to be jobs involving exposures near or above the Swedish exposure limit of 0.5 ppm. For whole-day monitoring measurements around such operations as composite board manufacture and glueing of plywood/veneers, 30% of the measurements were in the range 0.08 – 0.5 ppm (0.1 – 0.6 mg/m^3) and the others were lower. The report states that formaldehyde is not a major problem in working with laminates. Whole-day monitoring also showed low exposures, usually ≤0.024 ppm (≤0.03 mg/m^3), in machine shops where cutting fluid was in use. Formaldehyde was also monitored in pathology and cytology laboratories at hospitals. Low exposure levels were recorded, which may be due to the use of 4% formalin in these workplaces. Most of the whole-day measurements showed levels of <0.05 ppm (0.06 mg/m^3). The highest reported whole-day average was 0.12 ppm (0.15 mg/m^3). The highest short-term values measured, 0.92 ppm (1.14 mg/m^3, 35 minutes) and 0.84 ppm (1.04 mg/m^3, 30 minutes), were around emptying buckets of used formalin into a waste sink.

Inhalation exposure to formaldehyde in contexts other than occupational exposure can occur with exposure to cigarette smoke and vehicle exhaust, as well as emissions from construction materials, furniture, water-based paints, textiles etc. Other indoor sources are food preparation and open fires (11). Results from personal monitors in Sweden during the past few years have shown average levels around 0.01 – 0.025 ppm (13 – 31 μg/m^3, range 6 – 94) in groups without

occupational exposure (11). It is reported than in smoking a pack of cigarettes a day a smoker can inhale 0.4 – 2 mg formaldehyde (58).

Uptake, biotransformation, excretion

Formaldehyde in aqueous solution can be absorbed through the skin. In *in vitro* tests with human skin, the rate of uptake for a concentrated formalin solution (37%; methanol content 10 – 15%) was 319 μg/cm^2/hour, and for a 3.7% formaldehyde solution in phosphate buffer (containing 1 – 1.5% methanol) was 16.7 μg/cm^2/hour (58, 79). This large difference may be due to the methanol additive, which is known to accelerate uptake (64).

Uptake with inhalation exposure is high, and because the substance is water-soluble it is absorbed in the upper respiratory passages. Adsorption of formaldehyde on dust particles was studied to assess the extent to which such particles can function as carriers. The dose of particle-borne formaldehyde that reaches the lower respiratory passages was estimated to be at least 10,000 times lower than the dose in gas phase that reaches the upper respiratory passages (58). Adsorption of formaldehyde on inhaled particles thus has little effect on the deposition pattern (in comparison to formaldehyde gas) (58). It has been shown in animal studies that formaldehyde in inhaled air disappears completely, and on the basis of this observation uptake has been assumed to be 100% (11, 32). Some formaldehyde, however, dissolves in the mucus layer, reacts with components in the mucus, and is carried away by mucociliary clearance, which protects the underlying epithelium (14, 58). This protective function is less effective at higher air concentrations. In an inhalation study with rats, severe inhibition of mucociliary clearance in the nose was observed at 15 ppm, much less inhibition at 6 ppm, minimal inhibition at 2 ppm and no such effect at 0.5 ppm (96). Free formaldehyde can form covalent bonds with DNA, nucleosides, nucleotides, proteins, amino acids, glutathione etc. and form adducts and DNA-protein crosslinks (DPC). Free formaldehyde can also be deactivated by metabolism in the mucous membranes of the respiratory passages (e.g in epithelial cells) or in the blood (20, 21, 22, 32, 80, 102).

Absorbed (exogenous) formaldehyde is rapidly metabolized in the blood (by erythrocytes). The half time in rat plasma after intravenous administration of formaldehyde is about a minute (58). The major metabolic pathways in the cells involve oxidation to formate (formic acid salt), but formaldehyde can also be reduced to methanol. Formaldehyde is metabolized to formate mostly by formaldehyde dehydrogenase (ADH3) or directly via aldehyde dehydrogenases (ALDH1A1, ALDH2). In the first case, the formaldehyde reacts spontaneously with glutathione (GSH), forming S-hydroxymethyl glutathione, which is oxidized by ADH3 to S-formyl glutathione. S-formyl glutathione is subsequently metabolized by S-formyl glutathione hydrolase, producing formate. Formate is transformed to carbon dioxide and water, which can be exhaled or eliminated via the kidneys along with formic acid. Formaldehyde/formic acid can also be "eliminated" by being added as fragments in the body's C_1 pool and incorporated

into other molecules. Formaldehyde is a normal, endogenous metabolite found in all types of cells and is an intermediary in biosynthesis of purines, thymidine and some amino acids (11, 32, 58, 80, 129).

The concentration of endogenous formaldehyde in human blood is 2 – 3 mg/liter (about 0.1 mmol/l). This figure represents the total concentration of both free and reversibly bound endogenous formaldehyde in blood. Similar levels occur in the blood of rats and monkeys (50, 58). Because of formaldehyde's deposition in the upper respiratory passages and rapid metabolism, it has been impossible to demonstrate any increase in blood levels even at air levels of several ppm (11, 58). Blood levels had not risen significantly in monkeys after 4 weeks of exposure to 6 ppm (6 hours/day, 5 days/week) or in rats after inhalation of 14.4 ppm for 2 hours (50, 58). Six volunteers (nonsmokers) were exposed to 1.9 ppm for 40 minutes: the average formaldehyde level in blood was 2.61 µg/g prior to exposure and 2.77 µg/g immediately afterward (the difference is not significant). For some subjects, formaldehyde levels in blood were significantly higher after the exposure than before it, while for others they were significantly lower (50). The average level of formate in urine of a group of students was reported to be 12.5 mg/l, with large inter- and intra-individual variations. No significant changes in formate level were reported after exposure to up to 0.36 ppm formaldehyde for a 3-week period (urine samples were taken within 2 hours after the end of exposure) (40).

In a recently published work (80), the adduct S-(1-(N^2-deoxyguanosinyl)methyl) glutathione is proposed as a specific biomarker for formaldehyde exposure. Isotope labeling of formaldehyde in exposure studies makes it possible to differentiate specific adducts of this type from the same adducts formed by endogenous formaldehyde exposure (80). These authors also report that with inhalation exposure to isotope-labeled formaldehyde, the isotope can be traced in the DNA adduct N^2-hydroxymethyl-deoxyguanosine in nasal mucosa (81).

Toxic effects

Animal data

Effects on respiratory passages

Sensory irritation with short-term exposures, expressed as RD_{50} (50% reduction in respiratory rate), has been reported to be 10 – 30 ppm for rats and about 3 – 5 ppm for mice (31, 33). The RD_{10} for mice was reported to be 0.3 ppm, near or at the NOEL (no observed effect level) (31).

In a recent inhalation study (4), rats were exposed to 0.6, 1.8 or 5 ppm formaldehyde 6 hours/day, 5 days/week for up to 3 weeks (one portion of the study), and cell proliferation in nasal mucosa and other changes in the nose were examined. The histopathological examination revealed changes chiefly in respiratory epithelium and transitional epithelium, especially inflammatory cell infiltration, epithelial hyperplasia and epithelial squamous metaplasia. It was reported that changes definitely related to the treatment occurred only at 5 ppm, although an elevated incidence of epithelial hyperplasia was also seen at 1.8 ppm. Minimal

inflammation was seen in all exposure groups (including controls), but consistent increases of frequency and degree were observed only at 5 ppm. Cell proliferation was assessed on days 5 and 15. A significant increase of cell proliferation in nasal epithelium was observed at 5 ppm on day 5, but both degree and extent had decreased on day 15. Analysis showed no significant changes at 0.6 ppm. Minimal changes in gene expression were seen at 1.8 ppm, but changes in several different genes were observed at 5 ppm.

Numerous animal studies with chronic and subchronic inhalation exposure to formaldehyde have been published (32, 33, 58). A review made by TNO (Netherlands Organization for Applied Research) reports that slight effects on nasal mucosa (hyperplasia/metaplasia in respiratory epithelium) were seen in rat studies at air levels of about 2 – 3 ppm, and more pronounced effects of the same type, as well as cell necrosis and severe inflammation of nasal mucosa, were usually observed at levels ≥6 ppm. No hyperplasia/metaplasia was reported at 1 ppm, which was given as the NOAEL (no observed adverse effect level) for nasal damage (7).

In a comparative inhalation study with rats, it was shown that the effects on respiratory passages were dependent on the concentration rather than the total dose of formaldehyde (142). Significant increases in degree and frequency of histopathological changes in nasal respiratory epithelium (including focal squamous metaplasia) were seen with intermittent exposure to 4 ppm, 4 hours (8 x 30 minutes)/day, 5 days/week for 13 weeks, but not with continuous exposure to 2 ppm 8 hours/day for the same period (142). There are also indications that the time available for repair and recovery may affect the degree and extent of damage to nasal mucosa (7). The survey article by Arts *et al.* (7) reports that definite toxicity (including cell necrosis, extensive hyperplasia/metaplasia) is usually observed at exposure levels of 6 ppm or higher, but in the study by Cassee and Feron (23), with 3 days of intermittent 8-hour exposures to 3.6 ppm separated by only 4 hours, both degeneration and necrosis, as well as hyperplasia/squamous metaplasia, were observed in respiratory epithelium of the exposed rats along with moderate to marked inflammation of nasal mucosa. In a study in which monkeys, rats and hamsters were exposed almost constantly to 0.2, 1 or 3 ppm formaldehyde for 26 weeks, biologically significant changes were seen at 3 ppm in rats and monkeys, but not hamsters. In the rats, the observed effects on respiratory passages took the form of elevated occurrences of inflamed nasal mucosa, squamous metaplasia/hyperplasia, and basal cell hyperplasia in nasal conchae at 3 ppm, and in the monkeys elevated incidences of nasal secretion, hoarseness and in 6/6 animals squamous metaplasia/hyperplasia in nasal conchae. At 1 ppm squamous metaplasia/hyperplasia (nasal conchae) was found in 1/6 monkeys (109). In the study by Zwart *et al.* (153), in which rats were exposed 6 hours/day, 5 days/week, squamous metaplasia was seen in respiratory epithelium in a limited area in the outer part of the nose after exposure to 3 ppm formaldehyde for 13 weeks, but was not seen after exposure to 0.3 or 1 ppm on the same schedule.

In a 2-year study in which rats and mice were exposed to 2, 5.6 or 14.3 ppm formaldehyde 6 hours/day, 5 days/week, significant treatment-induced damage

was limited to the nasal cavity and the upper part of the trachea. In the rats, there were concentration-dependent increases (≥2 ppm) in frequency, severity and extent of inflammation in nasal mucosa, and epithelial dysplasia (basal cell hyperplasia) and squamous metaplasia in respiratory epithelium in the nasal cavity. At 2 ppm these changes were limited to the area furthest out in the nose. After 24 months the frequency of squamous metaplasia was near 100%. Mice showed clear irritation-induced effects at 14.3 ppm, although the effects could also be seen at 5.6 ppm. Focal atrophy of olfactory epithelium was observed in the mice at 5.6 ppm and 14.3 ppm (66). In another 2-year study with exposures to 0.7, 2, 6, 9.9 or 15 ppm, no formaldehyde-related effects are reported in the rats at 0.7 or 2 ppm. At 6 ppm there was minimal focal squamous metaplasia in the nasal cavities of the rats, and in the two highest exposure groups the effects in the nasal cavity included epithelial hypertrophy/hyperplasia, squamous metaplasia and inflammatory changes (95). (See also under the heading Cancer.)

Kamata *et al.* (65) exposed rats to 0.3, 2 or 15 ppm formaldehyde 6 hours/day, 5 days/week for 28 months, and observed significant and dose-dependent increases in incidence of squamous metaplasia, both with and without epithelial cell hyperplasia, in the nasal cavity at air levels ≥2 ppm. At 15 ppm there was also a significantly increased incidence of epithelial cell hyperkeratosis (nasal epithelium). In a study in which rats were exposed to 0.1, 1 or 9.2 ppm for 3 months with a subsequent observation period of 25 months, or to 0.1, 1 or 9.8 ppm for 28 months, formaldehyde-related effects were observed in the noses of animals with undamaged nasal mucosa only at 9.2 and 9.8 ppm (see Table 3). Animals with deliberately damaged (by electrocoagulation) nasal mucosa had higher incidences of squamous metaplasia in respiratory epithelium and inflammation in nasal mucosa at air levels ≥0.1 ppm, and basal cell hyperplasia in respiratory epithelium at ≥1 ppm (28-month exposure) (145). (See also under the heading Cancer.)

Hyperreactivity in respiratory passages after formaldehyde exposure was studied in guinea pigs (125). The animals were exposed to 0.86, 3.4, 9.4 or 31.1 ppm formaldehyde for 2 hours, or to 0.11, 0.31, 0.59 or 1.05 ppm for 8 hours. Bronchial constriction, expressed as higher specific total airway resistance (sR_t) (reversible within 1 hour) was seen immediately after 2 hours of exposure to ≥9.4 ppm or 8 hours of exposure to ≥0.31 ppm (significant at 1.05 ppm). Two hours of exposure to ≥9.4 ppm also reduced the effective dose of acetylcholine (infusion) that doubled the specific airway resistance at rest (ED_{200}) (significant 2 to 6 hours after exposure). Eight hours of exposure to 1.05 ppm also had this effect, and was significant compared to controls even 24 hours after the exposure. DECOS/NEG (32) concluded that the NOAEL for bronchial reactivity in guinea pigs is 0.11 ppm formaldehyde.

Irritation and adjuvant effects of formaldehyde were examined in a rat study: 5 groups of rats were exposed to saline, ovalbumin (OVA), OVA + 0.4 ppm formaldehyde, OVA + 2.5 ppm formaldehyde, or 2.5 ppm formaldehyde without OVA immunization. OVA exposure was by intraperitoneal injection on days 10 and 18 (dose not reported) and by inhalation on days 22 – 28 (30 minutes of

provocation with 1% OVA aerosol). Formaldehyde exposure was on days 0 – 21 (0.4 ppm or 2.5 ppm, 6 hours/day). The negative controls were given only saline. Methacholine was used to measure bronchial hyperreactivity. The formaldehyde exposure increased the degree of bronchial reactivity (dose-dependent) in the OVA-immunized animals (compared to animals exposed only to OVA). Animals exposed only to formaldehyde (2.5 ppm) showed greater bronchial reactivity than the negative controls but less than the formaldehyde-exposed OVA groups. The degree of structural changes in the lungs (histological examination) of the OVA-immunized animals also rose with formaldehyde exposure, especially at 2.5 ppm, whereas only slight (not significant) changes were observed in the lungs of animals exposed to formaldehyde alone (2.5 ppm). To assess the inflammatory effects of formaldehyde, the cytokine levels (IL-4, IFN-γ) in lungs and the numbers of eosinophilic cells in bronchoalveolar lavage were quantified. IL-4 (significant at 2.5 ppm) and eosinophils (significant at 0.4 and 2.5 ppm) were higher (dose-dependent) in the OVA-immunized rats exposed to formaldehyde than in the OVA-immunized rats not exposed to formaldehyde, while IFN-γ was not significantly affected. IFN-γ was significantly elevated and IL-4 significantly reduced (no significant effect on number of eosinophils) in animals exposed only to formaldehyde (2.5 ppm), compared to negative controls. The authors give several possible mechanisms for development of formaldehyde-related asthma. It was shown that formaldehyde alone (2.5 ppm) can function as an irritant and increase bronchial reactivity, which may be involved in the etiology of asthma (108). Studies with mice indicated that 5 weeks of exposure to 0.8 ppm formaldehyde, but not 0.1 ppm (24 hours/day, 5 days/week) increases OVA-specific IgE (but not IgG) antibody formation ($p<0.05$) in animals sensitized with OVA allergen (sensitization by intraperitoneal injections of 10 μg OVA on days 0 and 7). Formaldehyde alone (in animals not sensitized with OVA allergen) induced neither IgE nor IgG antibodies specific for formaldehyde during the exposure period (41).

Other effects

Few effects other than those on respiratory passages have been reported in animal studies with inhalation exposure to low levels of formaldehyde. Özen *et al.* (154) reported significantly higher levels (dose-dependent) of zinc and copper and somewhat lower levels of iron ($p<0.05$ at 9.8 ppm) in the cerebral cortex of rats after exposure to 4.9 or 9.8 ppm (6.1 or 12.2 mg/m^3) 8 hours/day, 5 days/week for 4 or 13 weeks. Dose-dependent declines in weight gain ($p<0.001$) were also recorded. A small study with mice (82) reports effects on the central nervous system. The animals (5 per dose group, males only) were exposed to 0.8 ppm (1 mg/m^3) or 2.4 ppm (3 mg/m^3) 6 hours/day for 7 days. Behavior tests (Morris water maze, with or without a platform in the pool) were given daily 30 minutes after the exposure ended. Clear and significant ($p<0.01$) effects on spatial learning and memory were observed at 2.4 ppm (the animals were described as confused), but no significant effects were seen at 0.8 ppm. Superoxide dismutase (SOD),

malondialdehyde (MDA) and glutathione (GSH) in the brain were measured immediately after the final test, and significant effects on all three parameters were observed at 2.4 ppm. MDA was increased and SOD and GSH were reduced, which the authors attributed to neuron damage induced by oxidative stress. Significantly elevated gene expression of N-methyl-D-aspartate (NMDA) receptors (NR1 and NR2B mRNA) in the brain was also seen at 2.4 ppm, and it was suggested that this might be a compensatory mechanism for inhibited synapse transmission. According to the authors, the study indicates negative effects on learning and memory at 2.4 ppm but not at the lower air level. A briefly described new study (131) reports that decline of spatial learning/memory was observed in mice given 0.5 mM formaldehyde intraperitoneally for 30 days and then given the behavior test (Morris water maze with or without a platform in the pool), in comparison with a control group injected with a physiological saline solution. After the final behavior test the animals were killed, and elevated levels of formaldehyde were found in the brain ($p<0.01$) (compared to controls). Injection of formaldehyde with resveratrol (antioxidant, vegetable polyphenol) was reported to somewhat reduce the negative effects on the test results.

Behavioral effects are also reported in a small study with rats (maze test, 13 animals per dose group). Exposures were 2.6 or 4.6 ppm, 10 minutes/day, 7 days/week for 90 days, followed by 1 month of observation. Tests were initially given weekly and later at ten-day intervals (at least 22 hours after the most recent exposure, to avoid effects on olfactory organs). The number of mistakes was significantly higher in week 12 of the exposure period, but there was no significant difference between 2.6 and 4.6 ppm. From week 7 onward the exposed rats took significantly longer than controls to complete the test, but here also a dose-response relationship was lacking. The behavioral effects were still seen to some extent in tests given during the observation period. The authors report that no indication of impaired movement was seen in any group. Histological examination (optical microscope) of various parts of the brain and spinal cord revealed no changes. On the basis of their study results, the authors classified formaldehyde as “probably neurotoxic” (106). In another study, with exposures of 0.1, 0.5 or 5.4 ppm 2 hours/day for 10 days, the rats were tested daily, 2 hours after exposure, with a behavior test used to study memory and learning. After 5 or 6 days of exposure to 5.4 ppm the rats consistently took a significantly longer time than controls to swim through a labyrinth. On several days there was also a significant increase at 0.5 ppm. Significant increases in mistakes (swimming in the wrong direction, swimming in circles) were seen after a few days of exposure at all air levels, compared to controls, but there was no clear dose-response relationship. The authors concluded that the formaldehyde exposure in the study affected learning and memory (85). Incompletely described methods and weaknesses in the statistical analyses (including numerous comparisons and risk of random significances), however, make the study extremely difficult to assess, and no dose-response estimate can be made for any part of the study. The reason for the effects

observed in these two studies (85, 106) is not clear. Effects on vision and olfaction due to some form of irritation has been suggested as a possible cause (58).

Reduced weight gain was recorded with exposure to 9.2 ppm in a 13-week study with rats. Poor weight gain has also been noted with long-term exposure to 5.6 ppm and 1 ppm. The effect at 1 ppm is difficult to explain, however, since somewhat higher body weights than controls were seen with the same air level and exposure schedule in rats with deliberately damaged nasal mucosa (66, 145). No other systemic effects were observed in these studies. In the study by Rusch *et al.* (109), with nearly constant exposure to 3 ppm for 26 weeks, exposed rats had poorer weight gain and lower absolute and relative liver weights than controls. Woutersen *et al.* (144) reported that no relevant differences in hematological parameters (Hb, hematocrit, numbers of erythrocytes, total leukocytes and different types of leukocytes) or urine parameters (including pH, protein, glucose) were seen in rats exposed to 0, 1, 10 or 20 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks. Nor were values for albumin, urea, creatinine and glucose in plasma either dose-dependent or significantly different from controls. Further, no differences were observed in relative liver weight or GSH in the liver. Activity of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkalic phosphatases in plasma, however, was significantly higher at 20 ppm, but only in males. At 20 ppm there was also significantly poorer weight gain in both sexes, as well as behavioral disturbances (ataxia, excitation) during the first 30 minutes of each exposure period. Somewhat poorer weight gain was also observed in the males at 10 ppm.

In another study, significantly elevated lipid peroxidation (measured as MDA) was noted in rat plasma ($p<0.05$) and liver ($p<0.05$) at 10.1 ppm (not significant at 5.1 ppm). Exposures were 0, 5.1 or 10.1 ppm, 6 hours/day, 5 days/week for 2 weeks. A significant increase in protein oxidation (plasma, liver) was also seen at 10.1 ppm (increase in plasma not significant at 5.1 ppm). A total of 32 proteins in plasma were identified by proteomic analyses as either upregulated or down-regulated (fibrinogen-γ chain precursor was one of those downregulated). In plasma, the cytokine IL-4 was upregulated and IFN-γ downregulated (dose-dependent), which was attributed to an inflammatory effect (61). Söğüt *et al.* studied biochemical effects on rat liver at 10 and 20 ppm (8 hours/day, 5 days/week for 4 weeks). Significant reductions in GSH concentration (dose-dependent) and xanthine oxidase activity (at 20 ppm) were recorded. Nitric oxide concentration and myeloperoxidase activity were the same as in controls, and MDA levels were lower (not significant) (126). Effects on the heart (rats) were studied at the same air levels (8 hours/day, 5 days/week for 4 or 13 weeks). Superoxide dismutase (SOD) activity in heart tissue was significantly higher in all exposed groups, and catalase activity was reduced in all exposed groups (significant only after 4 weeks). No significant differences were seen for lipid peroxidation (measured as MDA) or nitric oxide levels (42).

Whole-body exposure of mice and rats to 0.5, 1, 2, 6, 10 or 15 ppm formaldehyde, 6 hours/day, 5 days/week for 28 days, was followed by study of

nasopharynx-associated lymphoid tissues (NALT) and lymph nodes in upper respiratory passages. No significant effects were reported for the mice. The only distinct effects of the exposure observed in the rats were hyperplasia of the lymphoepithelium of NALT, associated with reduced cellularity (reduced numbers of lymphocytes) in some of the animals, at 15 ppm. (Reduced cellularity was also noted in 1 of 8 rats at 2 ppm, but without indications of epithelial hyperplasia, and the causative relationship to formaldehyde exposure is unclear) (38).

Contact allergy

Several sensitization studies with mice and guinea pigs have shown that formaldehyde is a potent contact allergen. In the Local Lymph Node Assay (LLNA) the EC_3 value is <1%, and in the Guinea Pig Maximization Test (GPMT) >60% of animals were sensitized (12). In guinea pig studies with formaldehyde in saline or water and multi-dose design, the maximum proportion of sensitized animals was estimated to be about 80 – 85% in the GPMT and 60% in the Buehler test (2, 3, 35).

Human data

Irritation effects

As formaldehyde levels increase, the first symptom to appear is usually eye irritation, followed by nasal irritation (32). There are fairly large individual differences in tolerance to formaldehyde (103). In a survey from 1997, a large number of scientific articles on the irritative effects of formaldehyde were reviewed and summarized by an expert panel (the Industrial Health Foundation panel). The panel reports that most people do not experience eye irritation definitely related to formaldehyde exposure at levels below 1 ppm, and that in these cases there is some acclimatization. The examined studies of volunteer subjects were reported to indicate that moderate to severe irritation of eyes, nose and throat usually does not appear until the air level exceeds 2 – 3 ppm, and also that persons exposed to 0.3 ppm for 4 to 6 hours in chamber studies usually experienced about the same amount of eye irritation as persons exposed to pure air (103).

In a more recent survey made by TNO, results from published studies were further processed and analyzed. (References 70 and 104, reviewed below, were not included.) The survey reports that unexposed controls were lacking in many studies measuring subjective irritation, and that in general it was hard to judge whether symptoms such as eye irritation were due to the formaldehyde exposure if they were reported by less than 20% of the exposed subjects (background incidence). The authors also questioned whether the very low subjective estimates of irritation (e.g. eye irritation) given at low air levels reflect real irritation (and are thus relevant). The intensity of the perceived odor was mentioned as a probable modifying factor in the subjective assessments of irritation. Using a normalized scale, the authors noted that, in studies with volunteers, mild/slight but “not bothersome” eye irritation was reported at

formaldehyde levels of 1 ppm and above, mild/slight irritation of respiratory passages at levels ≥2 ppm, and mild/slight throat irritation at ≥3 ppm. They also report that levels below 3 ppm did not cause breathlessness or coughing. Benchmark dose calculations based on the studies by Andersen and Mölhave (1) and Kulle (71) were also presented in the survey. For example, a benchmark dose for "slight discomfort" based on the first-named study, and assuming a background incidence of 6.25%, is 0.24 ppm (95% confidence interval, 10% extra risk); assuming a background incidence of 12.5% the dose is 0.43 ppm. A benchmark dose based on the second study (71) (95 % CI) for 10% extra risk for mild ("not bothersome") eye irritation is 0.56 ppm – an exposure level also expected to yield about 3% extra risk for moderate ("bothersome") eye irritation. Calculated in the same way, the extra risk at 1 ppm is 30% for mild eye irritation and 9.5% for moderate eye irritation. The authors of the survey article (7) considered the latter to be the first toxicologically relevant effect in the study by Kulle (71).

Andersen and Mölhave (1) exposed 16 students (5 of them smokers) to 0.24, 0.4, 0.8 or 1.6 ppm formaldehyde for 5 hours. At the two lower concentrations the subjects felt no discomfort (eye irritation, irritation/dryness in nose or throat) for the first couple of hours, but then the irritation began to increase, and by the final hour of exposure it had reached a plateau ("slight discomfort"). Slight irritation was reported by 3/l6 at 0.24 ppm and by 5/16 at 0.4 ppm (Table 2). At the two higher exposure levels the irritation began to increase during the first hour and reached a plateau after about 3 hours, or even diminished (at 1.6 ppm). Nearly all (15/16 and 15/16) of the subjects reported irritation from exposure at these levels. On average the irritation was still considered slight, although the estimation points were a bit higher than at the two lower exposure levels. Some of the subjects also reported more than slight irritation (up to 40 – 50 on a 100-point scale) at 0.8 and 1.6 ppm (1). In the opinion of DECOS/NEG, this study is not well documented (32).

Kulle (71) found significant dose-response relationships between 3 hours of formaldehyde exposure and subjective estimates of eye irritation and odor perception, but not nose/throat irritation or results of lung function tests. The study subjects were 19 nonsmokers. Mild odor was reported by 4/10 at 0.5 ppm and by 5/19 at 1 ppm. Odor perception was the most sensitive marker. Slight eye irritation was reported by 1/19 exposed to pure air and by 0/10 exposed to 0.5 ppm. At 1 ppm slight eye irritation was reported by 4/19 and moderate eye irritation by 1/19. Corresponding figures for 2 ppm were 6/19 and 4/19, and for 3 ppm 5/9 and 4/9. There was no significant difference between 1 ppm and pure air, but the authors nevertheless judged the threshold for eye irritation to be between 0.5 and 1 ppm. On the basis of these data, the IARC states that eye irritation increased linearly at concentrations between 0.5 and 3 ppm (58).

Bender *et al.* (13) investigated eye irritation in volunteers exposed to 0.35, 0.56, 0.7, 0.9 or 1 ppm formaldehyde for 6 minutes (5 to 28 subjects per group). The time elapsed before the subjects noticed eye irritation was compared to the

response time for pure air (each subject was his own control). There were large variations in response time, but there was a significant trend to earlier response with increasing formaldehyde concentration. The difference compared to pure air was significant only at 1 ppm, but the authors believe that significance might have been seen at 0.7 and 0.9 ppm if the groups had been larger. Eye irritation was reported to be very slight at 0.35 – 0.9 ppm (not ranked at 0 exposure). The authors consider their data to be in agreement with other studies in which eye irritation is reported at 0.4 – 1 ppm.

Both objective measurements of eye irritation (blinking frequency) and subjective estimates of eye, nose and throat irritation are reported in the study by Weber-Tschopp *et al.* (138). At about 1 ppm the average estimates of eye and nose irritation lay between "none" and "slight" but the authors report significant changes for eye and nose irritation at 1.2 ppm, and for throat irritation at 2.1 ppm. Blinking frequency (average values) was measured during the exposures, and significant increases were observed at 1.7 ppm and higher, but there were large individual differences. Exposure to 2.1 ppm caused doubled blinking frequency in 33% of the subjects, moderate eye irritation in 10% and severe or very severe eye irritation in 7%. At this level 20% of the subjects wanted to leave the exposure chamber.

In another exposure chamber study, 11 healthy subjects and 9 subjects with occupational exposure to formaldehyde were exposed to 0.4 ppm (0.5 mg/m^3) formaldehyde for 2 hours. All the occupationally exposed subjects were skin-sensitized to formaldehyde (positive patch tests), and reported temporary eye irritation, but not inflammation of nasal mucosa, at their workplaces. Prior to the exposure, and 10 minutes, 4 hours and 18 hours after the exposure, symptoms of nasal irritation – sneezing, itching, swollen mucous membranes etc. – were clinically evaluated (ranked on a scale) and the numbers of cells (epithelial, eosinophilic, neutrophilic, basophilic, mononuclear) and levels of albumin and total protein in nasal lavage were assessed. Symptoms of irritation appeared during the exposure but gradually disappeared afterward. Levels of inflammatory (eosinophilic) cells and protein (albumin, total protein) in nasal lavage were significantly above pre-exposure levels for up to 18 hours after the exposure. Sensitized and healthy subjects showed no differences in response to the formaldehyde exposure (104). In a later study (70), 10 healthy persons without occupational exposure to formaldehyde and 10 occupationally exposed persons who had inflamed nasal mucosa and asthma (assumed to be formaldehyde-related) were exposed to pure air or to an average 0.4 ppm (0.5 mg/m^3) formaldehyde (range 0.16 – 0.57 ppm; 0.2 – 0.7 mg/m^3) for 2 hours. Nasal lavage was performed before the exposure and 30 minutes, 4 hours and 24 hours afterward. All the subjects developed nasal symptoms (sneezing, itching, swollen mucous membranes) during the exposure. Increases in total leukocytes and eosinophilic leukocytes were noted immediately after the exposure, and were about the same in both groups of subjects. Elevated albumin/total protein ratios were also noted at that time, and interpreted as indications of increased permeability in nasal mucosa.

None of the asthmatics developed clinical symptoms of bronchial irritation during the provocation with formaldehyde. No significant changes in FEV_1, PEF or $PC_{20}H$ (histamine reactivity) values were seen in either the asthmatics or the healthy subjects after the provocation. According to the authors, these results indicate a non-specific, non-allergic inflammation process in nasal mucosa.

Lang *et al.* (73) exposed each of 21 test subjects to various concentrations of formaldehyde for ten 4-hour sessions, sometimes with ethyl acetate (EA) to mask the odor. Exposures were about 0.05 ppm (with or without EA), 0.15 ppm, 0.3 ppm (with or without EA), 0.5 ppm (with or without EA), 0.3 ppm with 4 exposure peaks of 0.6 ppm, and 0.5 ppm with 4 exposure peaks of 1 ppm (with or without EA). No significant formaldehyde-related effects on air flow, nasal air flow resistance or lung function were observed. Objective measures of eye irritation indicated effects only at 0.5 ppm with exposure peaks (the effects were described as minimal). Significant increases in average blinking frequency ($p<0.05$) were observed during exposures both with and without EA, but moderately (degree 3) reddened conjunctiva ($p<0.05$) were seen only with the exposures without EA (Table 2). Very slightly and slightly reddened conjunctiva (degrees 1 and 2) resulting from the exposures were not reported in the study. Subjective estimates of symptoms were also made, and all the participants considered them most severe after a bit over 3 hours. In exposures without EA, estimates of eye irritation and odor perception at both 0.3 and 0.5 ppm were significantly higher than at the near-0 exposure. Estimates of eye irritation were on average very low. At 0.5 ppm with exposure peaks the estimate averages were a bit higher, but eye irritation was still slight. For exposures without EA the average estimate of nasal irritation was significantly higher only at 0.5 ppm with peaks, but average estimates of respiratory irritation were significantly higher at 0.3 ppm without exposure peaks and 0.5 ppm with exposure peaks, though estimates were still very low. The authors concluded that eye irritation was the most sensitive effect parameter. When a personality variable was added as a covariant in the evaluations of subjective estimates, however, significance for eye irritation remained only for 0.5 ppm with peaks (with or without EA) and for exposure to 0.5 ppm with EA: 0.3 ppm was no longer seen as an effect level. Nasal irritation was significantly higher at 0.5 ppm with peaks (with and without EA). On the basis of both objective and subjective results, the authors gave 0.5 ppm with exposure peaks of 1 ppm as the LOEL (lowest observed effect level). The NOELs for eye irritation were considered to be constant exposure to 0.5 ppm, and 0.3 ppm with peaks of 0.6 ppm. The estimates of effect level took into account a possible effect of formaldehyde odor, influence of EA, and personality type (73). The authors' view that significance is lacking for subjective symptom estimates of eye irritation at 0.3 ppm can be questioned, however. The sample is small and correlations are weakened when another variable is added ("personality"). Further, there may have been some selection in that nervous or sensitive persons do not usually volunteer as test subjects. The Criteria Group therefore regards 0.3 ppm as the effect level for (very slight) eye irritation in this study.

In a Swedish study, 66 workers at a plant producing formaldehyde, who were said to be exposed to formaldehyde almost exclusively, reported on a questionnaire (not further described) more "workplace-related" symptoms involving eyes (20% vs. 0% in references), nose (53% vs. 3%, $p<0.001$) and lower respiratory passages (33% vs. 3%) than a reference group (n=36). A third (36%) of the formaldehyde-exposed workers had skin problems – eczema, itching etc. – compared to 11% in the reference group. Exposures of 0.04 to 0.5 ppm, with an average of 0.22 ppm (0.05 – 0.6 mg/m^3, average 0.26 mg/m^3), were recorded with personal monitors (141). This study has been described as poorly controlled and poorly documented (32).

Ballarin *et al.* (10) examined cells from nasal mucosa of 15 nonsmoking workers exposed to formaldehyde in a plywood factory and 15 matched controls. The reported formaldehyde levels (8-hour averages) were 0.07 – 0.3 ppm (0.09 – 0.39 mg/m^3). The workers also had low-grade exposure to wood dust (0.23 – 0.73 mg/m^3). Cytological examination revealed that the exposed group had chronic inflammation of nasal mucosa and a significantly higher frequency of squamous metaplasia than controls, but there was no dose-response relationship. Histo-pathological changes in nasal mucosa of workers exposed to formaldehyde have also been reported in several other older studies, but the air level at which these changes could have appeared is impossible to determine from these studies (33).

A study in which 150 medical students exposed to formaldehyde during dissections were compared to 189 matched controls reports significant differences in irritation of eyes, throat etc. The average formaldehyde concentration during a dissection was 0.74 ppm (range 0.4 – 1.2 ppm; personal monitors, 14 students). No differences were seen in lung function tests (average values for FEV_1 and FVC) given before and after a dissection session to 22 randomly chosen men and women (25).

Formaldehyde is one of the substances leading the list of suspected causes of eye, nose and throat complaints in indoor environments such as offices, but other factors may contribute to the symptom picture. Air levels of formaldehyde in these environments may be around 6 – 18 μg/m^3 (69, 111).

Asthma, allergy

The effect of formaldehyde on bronchi and lower respiratory passages has been explored in several studies in which asthmatics were experimentally exposed (11, 32, 58). In one study, asthmatics showed no significant changes (at the group level) in lung function or bronchial reactivity (although there were symptoms of eye, nose and throat irritation) on bronchial provocation with methacholine after exposure to 3 ppm (3.6 mg/m^3) for 3 hours (resting) (112). In another study (143) increased bronchial reactivity was noted on bronchial provocation with metha-choline, with a lower threshold dose in 8 of 12 asthmatics after 40 minutes of exposure to 2 ppm formaldehyde, but neither the average nor the median value for the group was significantly lower. Lung function was tested after 40 minutes of exposure to 2 ppm formaldehyde, but no consistent, significant changes were

observed. Harving *et al.* (44) exposed asthmatics to up to 0.7 ppm formaldehyde for 90 minutes without significant changes in lung function or bronchial reactivity (bronchial provocation with histamine). Cases of formaldehyde-related occupational asthma have been reported, however, and a small number of cases have been confirmed by bronchial provocation with formaldehyde (18, 52, 53, 99). The IARC states that the mechanism is probably hypersensitivity, since the reactions are often delayed, there is a latency period of formaldehyde exposure without symptoms, and asthmatics without earlier (occupational) exposure to formaldehyde do not react at the same concentrations. The IARC further states, however, that high formaldehyde levels can probably provoke asthma by an irritation mechanism, although it is unlikely that the substance provokes asthma in non-sensitized persons at levels ≤3 ppm (58). According to other authors, it has not been clearly shown that formaldehyde can sensitize respiratory passages (32, 70). Formaldehyde-specific IgE has seldom been found in connection with asthma, which indicates that formaldehyde does not work through IgE-mediated mechanisms. The proposed mechanisms include 1. that formaldehyde alone causes bronchial inflammation, or 2. that formaldehyde functions as an adjuvant in sensitization to common allergens (108).

Formaldehyde has sometimes been measured in studies of health complaints in home and school environments. Seven studies with 364 cases of pediatric asthma were combined in a meta-analysis (92). Six of the studies were of school-age children. They had been exposed at home, at school or outdoors. Measured air levels of formaldehyde vary between the studies, and none of them contains information on individual exposures. On the basis of all 7 studies, a 3% increase (95% CI 1.02 – 1.04; "fixed-effects model") or a 17% increase (95% CI 1.01 – 1.36; "random-effects model") in asthma risk was calculated for each increase of 10 $\mu g/m^3$ formaldehyde. When one of the studies is excluded, the OR in both cases is 1.24 (95% CI 1.09 – 1.42 for the "fixed-effects model"; 95% CI 1.07 – 1.44 for the "random-effects model") for each increase of 10 $\mu g/m^3$. (The children in the excluded study had an average age of <2 years.). The children with the highest exposure levels (80 $\mu g/m^3$; 7 studies) were reported to have a 3.5 times greater risk of developing asthma than unexposed children. The authors emphasized, however, that the correlation between asthma and the observed formaldehyde levels was uncertain. The risk of confounding in the pooled result was mentioned, and the authors pointed out the importance of prospective studies to investigate the connection between formaldehyde exposure and pediatric asthma (92). In some of the studies included in the meta-analysis, an elevated risk of asthma symptoms is also associated with other measured or observed air pollutants and/or environmental factors (sulfur dioxide, ozone, nitrogen oxide, volatile organic compounds (VOC), tobacco smoke, mildew, cat dander etc.). In the opinion of the Criteria Group, these studies support no definite conclusions regarding formaldehyde.

In a cross-sectional study with 99 exposed workers in a strand board factory and 165 controls from the petroleum industry, the workers exposed to formaldehyde

had significantly lower lung function ($FEV_{1.0}$/FVC) after correction for age and smoking habits. The OR was 3 (95% CI 1.1 – 8.1) for $FEV_{1.0}$/FVC <0.75 for exposed smokers compared to controls. The exposed group reported (questionnaire) significantly more symptoms involving the lower respiratory passages (corrected for smoking and age), and, consistent with that, more self-reported asthma. The concentrations of formaldehyde were 0.07 – 0.27 ppm (0.09 – 0.32 mg/m^3) in samples taken at 5 locations, and up to 50% of the formaldehyde came from particles. The average concentration of total dust (21 hours) around the saw line was 0.27 mg/m^3 and the particle size (MMAED, mass median aerodynamic equivalent diameter) was 2.5 μm. The authors state that the reported effects may be due to formaldehyde transported on particles to the lower respiratory passages (54).

Casset *et al.* (24) exposed asthmatics with mite allergy to 0.07 ppm (0.09 mg/m^3) formaldehyde or to a placebo (air; formaldehyde: 0.03 mg/m^3) via oral inhalation for 30 minutes. They were then given bronchial provocation tests with the mite allergen. There was a 20% reduction in FEV_1 with a lower induction dose of mite allergen (on average) and increased bronchial reaction (maximum FEV_1 reduction 15 vs. 11% within 6 hours) in subjects exposed to formaldehyde, compared to those exposed to air (Table 2).

Skin effects, contact allergy

Formaldehyde is a well known contact allergen and causes allergic contact eczema (74). Sensitization and eczema can be caused by chemical products, cosmetics and hygiene products, textiles and plastics. Formaldehyde and substances that emit formaldehyde are often used as preservatives in products that come into contact with the skin. Patch tests with formaldehyde given to eczema patients are about 3% positive in Sweden (75) and 2 to 4% positive in ten other European countries (132). In a Danish study, the prevalence of contact allergy to formaldehyde in the general population was found to be 1.7% (98).

Cardiovascular disease

Most of the occupational cohort studies that use national or regional cause-of-death statistics for comparisons report lower than average mortality due to cardiovascular disease. This is to be expected when a group of workers is compared to the total population, since the total population contains people who are unable to work because of heart disease. In some cohort studies using proportional mortality ratios (PMR), the PMR for ischemic heart disease was significantly above 1. Such an elevation in risk was seen for embalmers in California and New York (134) and for embalmers and funeral directors in the USA (48). Three studies divide their cohorts into different strata according to exposure. In a cohort of 25,619 persons working in industries that produced or used formaldehyde, the cohort could be divided into an exposed group and an unexposed group. The risk of death due to circulatory diseases was higher in the exposed group (standardized mortality ratio, SMR, 0.88, 95% CI 0.85 – 0.91) than

in the unexposed group (SMR 0.77, 95% CI 0.72 – 0.83). The risk of death due to circulatory diseases was significantly elevated (SMR 1.21) for the group with a highest formaldehyde exposure of 2 to 4 ppm, but not for the group exposed to more than 4 ppm (SMR 1.04). Nearly half of the cohort (47%) were exposed to one or several other chemical substances (46). In a group of foundry workers in the American automobile industry, the workers exposed to formaldehyde had a somewhat higher risk of ischemic heart disease (SMR 0.97, 95% CI 0.82 – 1.14) than the other foundry workers (SMR 0.87, 95% CI 0.73 – 1.04) (5). In a British cohort of 14,014 persons who worked in companies that produced or used formaldehyde, the SMR for circulatory diseases was 0.98 (95% CI 0.94 – 1.02). The risk was somewhat higher for the subgroup with high (over 2 ppm) formaldehyde exposure (SMR 1.04, 95% CI 0.97 – 1.11) (26). These three latter studies (5, 26, 46) suggest a possible connection between exposure to formaldehyde and occurrence of cardiovascular disease. Two of the studies have very rough categorizations of these diseases. A more precise categorization of these diseases is needed, since in another cohort an elevated risk was found for another heart disease – "heart failure" (105). A better exposure categorization is also needed, to allow identification of any dose-response relationships between formaldehyde exposure and the occurrence of cardiovascular disease.

Other effects

A recently published prospective study (139) reports a not-significant increase in mortality due to amyotrophic lateral sclerosis (ALS) among persons with self-reported exposure to formaldehyde (RR 1.34, 95% CI 0.93 – 1.92). Exposed persons were also categorized by number of years of exposure (self-reported), and a strongly significant dose-response correlation (p for trend 0.0004) was observed. When persons who reported exposure, but not duration, were excluded from the analysis, the RR was 2.47 (95% CI 1.58 – 3.86, $p<0.0001$). The authors emphasize, however, that these findings should be interpreted with great caution and need to be confirmed by other studies (139).

A significant increase of B lymphocytes and reduction of certain T lymphocytes (CD3, CD4, CD8) was observed in anatomy students in a study by Ying *et al.* (148). The average exposure to formaldehyde during the anatomy lessons (3 hours) was 0.4 ppm (0.5 mg/m^3). Significant increase of B lymphocytes and reduction of T lymphocyte subsets (CD3, CD8) was also seen in a study of factory workers exposed to formaldehyde. The reported 8-hour average for exposure in this study was 0.8 ppm (1 mg/m^3); top exposure was 1.4 ppm (1.7 mg/m^3) (146).

An incomplete and not well controlled Chinese study (72) reports that numbers of white blood cells in a group of hemodialysis nurses exposed to formaldehyde were significantly lower than in a control group (nurses). Further, a survey article (127) states that reduced numbers of white blood cells have also been reported in several other Chinese studies of groups exposed to formaldehyde (industrial workers, pathologists), but no further details are given. The survey also mentions that lower Hb and reduced numbers of thrombocytes were documented in a few

of the studies. Since the original data are not available this information can not be evaluated.

However, effects on the blood cells of Chinese factory workers exposed to formaldehyde are also reported in a newly published study (151). Two factories where formaldehyde-melamine resin was produced or used, and without exposure to other substances that are known or suspected to affect blood profile/bone marrow (e.g. benzene) and 3 control workplaces without exposure to genotoxic or hematotoxic chemicals were studied. Subjects of the study were 43 workers with formaldehyde exposure who had held their jobs for at least 3 months and 51 matched controls from the same geographic area. The median formaldehyde level (personal monitors, 8-hour time-weighted average) for the 43 exposed workers was 1.28 ppm, with 0.63 ppm for the 10th percentile and 2.51 ppm for the 90th percentile. Hematological examination showed that the total number of white blood cells in blood from the exposed workers (both factories) was significantly lower than in controls (p 0.0016): the number/µl blood was reduced by 13.5%. Exposed workers also had significantly lower numbers of lymphocytes (p 0.0002), granulocytes ($p<0.05$), thrombocytes ($p<0.05$) and red blood cells ($p<0.001$), as well as elevated ($p<0.05$) MCV (mean corpuscular volume, a measure of red blood cell size). Proliferative potential of myeloid progenitor cells from blood samples was determined *in vitro,* and 20% lower growth was observed in blood from the formaldehyde-exposed workers (p 0.10). According to the authors, this indicates that formaldehyde has a possible toxic and/or inhibitory effect. The effect of added formaldehyde on stem cells in human blood was also examined. Mononuclear cells from a person were used, and the generated colonies of different stem cells were studied. All three types of studied stem cells decreased in number with increasing formaldehyde concentration (100 – 200 µM), and for the most primitive stem cells there was a linear negative dose-response relationship. Primitive stem cells of this type were reported to be target cells and to be transformed to leukemia stem cells in acute myeloid leukemia. The study also reports the result of cytogenetic examinations made to map the frequency of chromosome aberrations typical of myeloid leukemia and myelodysplastic syndrome in a subgroup of highly exposed workers. These results are reviewed below in the section on mutagenicity.

Mutagenicity, genotoxicity

In vitro

Formaldehyde has been shown to be mutagenic/genotoxic in numerous *in vitro* test systems and in *in vivo* studies with Drosophila. It is well known that the substance is mutagenic in test systems with bacteria and lower eukaryotes and clastogenic in mammalian cells. Reaction with DNA *in vivo* gives rise to several formaldehyde-DNA adducts and crosslinks, e.g. the crosslinked adduct di-(N^6-deoxyadenosyl)methane (dAdo-CH_2-dAdo) and its precursor, the hydroxymethyl adduct N^6-hydroxymethyl-dAdo (also called N^6-hydroxymethyl-deoxyadenosine;

N^6-HOMe-dAdo) (135, 136). Formaldehyde can also bind glutathione (GSH) to DNA by formation of S-(1-(N^2-deoxyguanosinyl)methyl) glutathione via the reactive intermediary S-hydroxymethyl glutathione (80).

In studies with human cells *in vitro*, formaldehyde has been shown to induce DNA-protein crosslinks (DPC), sister chromatid exchanges (SCE), chromosome aberrations (CA) and gene mutations (58, 102). DPC is the predominant form of DNA damage in formaldehyde-exposed cells and has been called "the primary" DNA change (58, 102, 113). DPC have been shown *in vitro* with clear dose-effect relationships. It has been reported that crosslinks of this nature are efficiently repaired (with a half time of 3 to 66 hours), but in the presence of unrepaired or incompletely repaired DPC other genotoxic/mutagenic effects such as SCE, chromosome aberrations and micronuclei can be induced (58, 113). Schmid and Speit (113) studied dose-response for formaldehyde genotoxicity in whole human blood, and found that DPC are induced at concentrations ≥25 μM. At 100 μM, DPC had completely disappeared after 8 hours (prior to DNA replication of lymphocytes), whereas some of the crosslinks induced at 200 or 300 μM were still present after 24 hours. SCE were induced at 200 μM, and at this concentration there was significant cytotoxicity. Some (not significant) cytotoxicity could also be observed at 100 μM. Micronuclei were induced under special conditions, with significant induction at concentrations ≥300 μM, and were reported to be of the clastogen type.

In another *in vitro* study (77), using peripheral lymphocytes from human blood and HeLa cells, no significant increase of DPC was seen at formaldehyde levels below 50 μM, although an increase of single-strand breaks in DNA (DSSB) was seen at lower doses (<30 μM). DSSB, however, were repaired much more rapidly than DPC, with complete repair after 90 minutes. It was also suggested in the study that DNA-DNA crosslinks could be induced at doses >25 μM. In studies with human leukocytes, Frenzilli *et al.* (37) found that formaldehyde induced DNA crosslinks at levels ≥400 μM, and DNA strand breaks at concentrations ≤50 μM. DNA strand breaks at low formaldehyde concentrations (5 – 10 μM) have also been observed in several studies with other types of cells (77).

Animal data

In a new study (81), rats were exposed by inhalation to 10 ppm isotope-labeled formaldehyde 6 hours/day for 1 or 5 days, and tissue content of DNA adducts (including N^2-hydroxymethyl-deoxyguanosine) was analyzed. The analysis differentiated exogenous adducts from endogenous adducts by using formaldehyde labeled with carbon 13 and deuterium ($^{13}CD_2$-formaldehyde). Equal levels of endogenous adducts were found in several organs, including nasal mucosa, blood, spleen, thymus and bone marrow. Exogenous adducts were found only in nasal mucosa, and there usually in lower amounts than the endogenous adducts. It was concluded from this observation that formaldehyde is unlikely to be genotoxic in tissues other than the "first-contact tissue" and that the data support a nasal carcinogenicity effect. The data were also considered to strengthen arguments for

the role of cytotoxicity-induced proliferation in mutagenicity and the occurrence of cancer, and also against the likelihood that inhaled formaldehyde causes leukemia. The authors point out, however, that extrapolating from one species to another can be misleading (81). In the opinion of the Criteria Group, this study does not exclude the possibility that hematopoietic stem cells in mucous membranes of the nose and throat will mutate there, in accordance with one of Zhang's hypotheses on the etiology of leukemia (see below). Mutated stem cells of this type can not be detected with the method used by Lu *et al.* (81), but according to Zhang *et al.* (150) they can cause leukemia. Arguing against Lu's conclusions, in addition to the leukemia studies, are also the observations of DNA adducts and micronuclei in blood in several human studies (see below under *Human studies*).

Meng *et al.* (93) studied the origins of two “hot spot” mutations (commonly occurring with tumors) in the p53 and ras genes in the nasal mucosa of rats exposed to up to 15 ppm formaldehyde for 13 weeks. Some p53 mutations were found in controls but there was no increase in the exposed rats, although at 15 ppm cell proliferation had more than doubled. It was concluded that the p53 mutations observed in rat tumors in earlier cancer tests appeared late in tumor development.

In studies with rats and monkeys, DNA-protein crosslinks in respiratory passages have consistently been observed after 3 to 6 hours of inhalation exposure (58). In rats, DPC in nasal respiratory epithelium have been observed at air levels ≥0.3 ppm (20). In monkeys, DPC in nasal mucosa have been observed at air levels ≥0.7 ppm, and the concentration was highest in the middle turbinates (21). Non-linear increases of DPC have been reported in both species with 6 hours of exposure to 0.3, 0.7, 2, 6 or 10 ppm (rats) and 0.7, 2 or 6 ppm (monkeys). The concentration-response curve for formation of crosslinks was biphasic, with increased steepness around 2 – 3 ppm for the rats. Similar results were seen for the monkeys, though the dose-response curve was less well defined (20, 21, 58). No significant increase of DPC in bone marrow was seen in studies in which rats were exposed to 0.3 – 15 ppm formaldehyde for 3 to 6 hours (19, 22, 58).

The results of other animal experiments (mice, rats) reviewed by the IARC, with other genetic endpoints, are mixed and harder to assess. Chromosome-damaging effects have been examined with micronuclei tests in some *in vivo* studies, but in only one study with rats, with single oral doses of 200 mg formaldehyde/kg body weight and registration of micronuclei in epithelial cells of the digestive tract, was the result positive. The greatest effect was observed in the stomach, with a 20-fold increase of micronucleated cells 30 hours after the dose, and the least effect was observed in the large intestine. The frequency of nuclear anomalies was also significantly elevated in the digestive tract. The observed effects were reported to be accompanied by indications of severe local irritation (94). The occurrence of chromosome aberrations and SCE has also been studied in various kinds of cells from mice and rats exposed by inhalation or intraperitoneal injection. Most of these studies were negative (58): for example, formaldehyde caused no increase of chromosome aberrations or SCE in lymphocytes in a study

in which rats were exposed by inhalation to up to 15 ppm formaldehyde 6 hours/day for 5 days (68). However, positive results were reported in a Russian study in which rats were exposed by inhalation to 0.4 or 1.2 ppm (0.5 or 1.5 mg/m^3) 4 hours/day for 4 months. Significant increases in the number of aberrations in bone marrow cells were observed at both 0.4 ppm (chromatid type) and 1.2 ppm (chromosome type), and the study is reported to show that formaldehyde is cytotoxic and mutagenic to bone marrow cells at the lower exposure level (Kitaeva *et al.* 1990, cited in Reference 58). The IARC report points out, however, that chromosome loss often occurs as an artefact and that the study needs to be repeated. In a study in which rats were exposed to 0.5, 3 or 15 ppm 6 hours/day, 5 days/week for 1 or 8 weeks, no significant increase of chromosome aberrations was observed in bone marrow cells, although at 15 ppm chromosome aberrations (mostly chromatid breaks) were significantly higher in alveolar macrophages after both 1 and 8 weeks (30).

A newly published rat study reports no induction of micronuclei, DNA strand breaks or DPC in alveolar cells after inhalation exposure to 0.5, 1, 2, 6, 10 or 15 ppm formaldehyde 6 hours/day, 5 days/week for 4 weeks. The authors, however, do not regard micronuclei tests using cells in bronchoalveolar lavage fluid as a validated method (97). No significant effects were observed at these exposures in genotoxicity tests on blood (micronuclei in peripheral blood, SCE in lymphocytes, DNA strand breaks and DPC in leukocytes) (118). Other studies, however, have reported significant, dose-dependent increases in DNA damage (measured as DNA single-strand breaks, comet assay) in lung cells, peripheral lymphocytes and liver cells of rats. The animals had been exposed to 5.1 or 10.1 ppm formaldehyde 6 hours/day, 5 days/week for 2 weeks (61, 120).

Some dominant lethal tests (a type of mutation test) were also reviewed by the IARC (58). Negative or weakly positive results have been reported in mice with single intraperitoneal injections of high doses of formaldehyde (20 or 50 mg/kg b.w.). A weakly positive result at 1.2 ppm in a dominant lethal test is also reported in the Russian study mentioned above (Kitaeva *et al.* 1990, cited in Reference 58). In another study, in which rats were given 0.125 – 0.5 mg/kg b.w. by intraperitoneal injection, dose-dependent effects were observed in 2 mutation tests: a dominant lethal test and a sperm head abnormality assay (101). A poorly described Chinese study reports a significant, inherited increase in the number of tandem repeats in DNA after formaldehyde exposure. (The mechanism behind the origin of this type of mutation is unknown, but it is assumed to appear when DNA replication is disrupted.) Mice were exposed to 0, 2, 20 or 200 mg/m^3 formaldehyde for 2 hours, and the mutations were seen in the offspring of those exposed to 20 mg/m^3 (16 ppm) and 200 mg/m^3 (160 ppm). The mutations were dose-dependent and inherited via the male (78).

Human data

The IARC (58) reviews some human studies reporting local genotoxicity (mucous membranes of the mouth and nose). The most important of these studies are reviewed here. In a study by Ballarin *et al.* (10), cells from nasal mucosa of 15 nonsmokers exposed to formaldehyde in a plywood factory (8-hour averages 0.07 – 0.3 ppm, 0.09 – 0.39 mg/m^3) and 15 controls matched for age and gender were examined for micronuclei. The frequency of cells with micronuclei was significantly higher in the exposed group than in controls, but there was no significant difference between the group with the highest exposure (working in a warehouse with average exposure of 0.3 ppm, range 0.17 – 0.5 ppm) and the other exposed subjects. Low-grade exposure to wood dust (0.23 – 0.73 mg/m^3) also occurred in the factory (the lowest average exposure was in the warehouse), and the IARC notes that exposure to wood dust may have contributed to the elevated incidence of micronucleated cells in the exposed group. It should be mentioned, however, that 5/6 person in the warehouse (and 4/9 in other departments) had higher frequencies of micronucleated cells than any of the 15 controls. Ying *et al.* (147) assessed the frequency of micronucleated cells from nasal and oral mucosa of 25 anatomy students (nonsmokers) both before and after 8 weeks of intermittent exposure (3 hours, 3 times a week). Significantly higher average frequencies were observed in both nasal and oral mucosa after the final exposure. The time-weighted average for formaldehyde exposure during the 3-hour anatomy lessons was 0.4 ppm (0.5 mg/m^3) (range 0.06 – l.04 ppm, 0.07 – 1.28 mg/m^3). Two other studies of subjects exposed to formaldehyde in pathology and anatomy laboratories also report group averages for micronucleated cells from respectively nasal and oral mucosa that were significantly higher than controls. One of the studies (with 23 subjects) reports that the elevated frequency of micronuclei in nasal mucosa did not significantly increase with duration of exposure and was not influenced by either age, gender or smoking habits – though the average frequency was significantly higher ($p<0.01$) in exposed smokers than in unexposed smokers (controls) (16). In the other study, variance analysis indicated that the elevated frequency of micronucleated cells in oral mucosa of the formaldehyde-exposed subjects (n=28) was affected by neither gender nor smoking habits (17). Air levels in both studies were reported to be in the range 2 – 4 ppm (16, 17).

Suruda *et al.* (121) found a significantly elevated frequency of micronuclei in epithelial cells from the buccal area of the mouth, but not in cells from nasal mucosa, in students who were intermittently exposed to formaldehyde from embalming fluid during a course. Samples were taken before the course began and again after 9 weeks of lessons in the embalming laboratory (each person was his own control). There was a 12-fold increase in the frequency of micronuclei in oral mucosa ($p<0.05$) after the course, but significant increases were observed only in the men. A dose-response relationship with increasing cumulative exposure was noted for the 22 exposed men (p 0.01), but not for the 7 women. The average exposure during the embalming lessons was 1.4 ppm (range 0.15 – 4.3), but

occasional peaks up to 6.6 ppm were reported. The calculated 8-hour average exposure for days with lessons in the embalming lab was 0.33 ppm (range 0.1 – 1 ppm). In its 1995 monograph (57), the IARC states that this study is inadequately reported and therefore could not be evaluated. Other authors (117) have commented that the reported micronuclei frequency in oral mucosa prior to the exposure (0.046/1000 cells) was extremely low in this study. In a later work, Titenko-Holland *et al.* (130) analyzed samples from some of these students with other methods. This study also reports a significant increase in frequency of micronuclei in cells from oral mucosa, but not nasal mucosa, and the type of micronuclei indicated that the underlying mechanism was chromosome breaks. A weak correlation (p 0.06) between cumulative exposure to embalming fluid (for the entire period) and elevated frequency of micronucleated cells in oral mucosa was reported.

Some studies that may indicate cytogenetic effects in peripheral lymphocytes of persons occupationally exposed to formaldehyde have also been reviewed by the IARC (58). Shaham *et al.* (115) reported that the average frequency of DPC in lymphocytes of personnel exposed to formaldehyde in pathology departments was significantly higher than in controls (after adjustment for age, gender etc.). The exposed subjects were divided into a high (average 2.24 ppm, range 0.72 – 5.6 ppm) and a low (average 0.4 ppm, range 0.04 – 0.7 ppm) exposure group on the basis of 15-minute exposure measurements, but there was no significant difference between them. In an earlier study (same air levels) it had been found that the frequency of SCE in lymphocytes was significantly higher in the exposed group than in controls (adjusted for age, gender etc.). No difference between the high and low exposure groups was seen, but both variables of SCE were somewhat higher in the subgroup of highly exposed smokers than in the subgroup of smokers with low exposure (114). In the opinion of the IARC, DPC and SCE in peripheral lymphocytes showed a correlation to exposure in the studies by Shaham *et al.* (114, 115), but it is not clear what relevance these endpoints have for assessing the carcinogenicity or mutagenicity of formaldehyde (58). He *et al.* (49) found that 13 students (nonsmokers) exposed to formaldehyde 10 hours/week during a 12-week anatomy course had a significantly higher average frequency of chromosome aberrations and micronuclei in peripheral lymphocytes than 10 controls. (Correlations between micronuclei and chromosome aberrations were observed at the individual level.) The exposed students also had a somewhat higher frequency of SCE ($p<0.05$). The average exposure during a dissection was 2.37 ppm. The IARC (58) points out that frequencies of micronuclei and SCE were unusually low in the controls and that both breaks and gaps were included in the chromosome aberrations, which makes it difficult to assess the results. Ying *et al.* (147) assessed the micronuclei frequency in lymphocytes from anatomy students, but averages were not significantly higher. The average formaldehyde level during an anatomy lesson (3 hours) was 0.4 ppm (0.5 mg/m^3). The authors report in a later study (148) that SCE in peripheral lymphocytes before and after the exposure (anatomy students, same exposure as above) were not significantly different.

Elevated frequencies of chromosome aberrations and SCE in peripheral lymphocytes have been seen in some older studies, but there are also some negative studies, and interpretation of these studies is reported to be difficult because of the contradictory results and the small numbers of subjects (56). Significantly ($p<0.05$) elevated frequencies of micronucleated lymphocytes in students intermittently exposed to formaldehyde during an embalming course are reported in the study by Suruda *et al.* (121, see above). Samples were taken before the course and again after 9 weeks of lessons in the embalming lab. No correlation to cumulative exposure was seen, however. Significant ($p<0.05$) reduction of SCE in lymphocytes was also reported. The IARC (57) considers this study to be inadequately reported and therefore impossible to assess.

In its summary, the IARC states that there is evidence that formaldehyde is genotoxic to humans and experimental animals. Studies of human subjects report elevated levels of DNA-protein crosslinks in workers exposed to formaldehyde. These observations are in agreement with animal studies demonstrating that formaldehyde exposure causes DNA-protein crosslinks in nasal mucosa of monkeys and rats. There is one study reporting abnormal cytogenetic effects in the bone marrow of rats after inhalation exposure to formaldehyde, but no other studies report any effects on bone marrow (58).

Studies not covered in the IARC monograph (58) and published in 2005 or later are presented in Table 1. In one study, cells from nasal mucosa of 18 formaldehyde production workers, 16 environmentally exposed waiters and 23 students (controls) were analyzed. The workers and waiters were on average somewhat older than the controls. Elevated group values ($p<0.05$) for frequencies of micronucleated and multimicronucleated cells were seen in the workers when they were compared to the control group, but no significant micronuclei induction was reported for the group of waiters. The 8-hour average exposure for the workers was 0.8 ppm (1 mg/m^3) and the 5-hour average for the waiters was 0.1 ppm (0.12 mg/m^3). Top exposures were 1.4 ppm (1.7 mg/m^3) for the workers and 0.24 ppm (0.3 mg/m^3) for the waiters (146). Speit *et al.* (117) analyzed cells from oral mucosa of 21 volunteers exposed to 0 – 0.5 ppm formaldehyde 4 hours/day for 10 days (different concentrations on different days), sometimes with 15-minute exposure peaks of 0.6 or 1 ppm (on some days the exposure included 10 – 20 ppm ethyl acetate). Cumulative exposure during the 10 days was reported to be 13.5 ppm-h. Samples were taken on two occasions before exposure, immediately after the 10-day exposure period, and 1, 2 and 3 weeks later. On each occasion 16 to 21 subjects were assessed. A somewhat elevated (not significant) average micronuclei frequency was noted immediately after the exposure period, but most of the increase could be traced to high frequencies in a few individuals. Analyses made 7 to 21 days after the exposure showed that the average micronuclei frequency gradually dropped (after 21 days it was lower than before the exposure).

Several more recent studies (see Table 1) have also reported positive results in micronuclei tests and other genotoxicity tests using peripheral lymphocytes from persons exposed to formaldehyde. Orsière *et al.* (102) examined genotoxicity in

lymphocytes from 59 workers in pathology and anatomy laboratories and 37 controls matched for gender, age and smoking habits. In the exposed group, no increase of DNA damage (measured as DNA repair ability) was seen in comparisons of measurements made before and after a workshift (1 day). The binucleated micronucleated cell rate (BMCR) was significantly higher than in the control group, but not correlated to either air levels or DNA damage. Special analyses of lymphocytes from a subgroup showed that the frequency of monocentromeric micronuclei (centromere-positive) was significantly higher than in controls (adjusted for age, gender etc.), which was regarded as an indication of an aneugenic effect. The authors suggest that these results may indicate formaldehyde-induced disruptions of the spindle function (mitosis) rather than a direct interaction with DNA in peripheral lymphocytes. The average formaldehyde exposure for the entire group was 2.0 ppm for 15-minute samples and 0.1 ppm for 8-hour samples; corresponding values for the subgroup were 2.3 and 0.1 ppm (60, 102).

Table 1. Recent genotoxicity studies of human subjects exposed to formaldehyde (not covered in the IARC monograph published in 2006, Reference 58).

Target organ	Endpoint	Result	Exposure (ppm)	Comments	Ref.
Nasal mucosa	Micronuclei	+ ($p<0.05$) (workers)	8-hr av. 0.8 peak 1.4	18 exposed workers, 16 waiters (low exposure), 23 controls; workers and waiters older than controls, sex ratio of waiters very different from the other two groups.	146
		- (waiters)	5-hr av. 0.09 peak 0.24		
Lymphocytes, peripheral blood	SCE	+ ($p<0.05$) (workers)	same as above		
		- (waiters)	same as above		
Lymphocytes, peripheral blood	DNA damage*	-	8-hr circa 0.1 15-min circa 2.0	57 workers in pathology and anatomy laboratories, before vs. after a workshift.	60, 102
	Micronuclei	+ (p 0.001) BMCR	8-hr 0.1 (<0.1-0.7) 15 min 2.0 (<0.1-20.4)	59 workers in pathology and anatomy laboratories, 37 controls; BMCR not correlated to either air levels or DNA damage.	
		+ (p 0.021) BMCR; + ($p<0.001$) C1+MN	8-hr 0.1 (0-0,3) 15 min 2,3 (0.4-7.0)	Subgroup: 18 exposed workers, 18 controls.	

Table 1. Continued.

Target organ	Endpoint	Result	Exposure (ppm)	Comments	Ref.
Oral mucosa	Micronuclei	-	Different concentrations on different days, 4 hrs/day, 10 days. Base exposure 0-0.5; some 15-min peaks of 0.6 or 1 (sometimes simultaneous exposure to 10-20 ppm ethyl acetate).	21 volunteers before vs. immediately after 10-day exposure and 1, 2 and 3 weeks later. (16-21 persons assessed on each occasion). Average increase seen only immediately after exposure period, elevated micronuclei frequencies in only a few subjects.	117
Lymphocytes, peripheral blood	Micronuclei	+ (p 0.003)	8-hr av. 0.44 (0.04-1.58)	30 workers in pathology and anatomy labs, 30 controls.	29
	SCE	+ (p<0.05)	same as above		
	Comet tail assay	+ (p<0.05)	same as above		
Lymphocytes, peripheral blood	Micronuclei	+ (p<0.01)	8-hr av. 0.83 (0.08-6.30) Subgroups:	151 workers in plywood factories, subgroups with 13-60 persons; 112 controls. (also correlation to duration of exposure)	63
		-	0.11 (0.08-0.15)		
		+ (p<0.05)	0.28		
		+ (p<0.05)	0.39		
		+ (p<0.05)	2.56 (0.81-6.30)		
	Comet tail assay	+ (p<0.01)	8-hr av. 0.83 (0.08-6.30) Subgroups:		
		+ (p<0.05)	0.11 (0.08-0.15)		
		+ (p<0.05)	0.28		
		+ (p<0.05)	0.39		
		+ (p<0.05)	2.56 (0.81-6.30)		
Myeloid stem cells, peripheral blood	Chromosome aberrations:		8-hr median 2.14 (1.38-4.14)**	10 highly exposed workers making or using formaldehyde-melamine resin, 12 controls.	151
	monosomy 7	+ (p 0.0039)			
	trisomy 8	+ (p 0.04)			
Lymphocytes, peripheral blood	Gene mutations (*HPRT*)	- ***	0.2-1 8-hr av. 0.7	21 women in 4 pathology labs, 37 controls.	62
	DNA repair	-			
	Total CA	+			
	Gaps	+			
	Aneuploidy	-***			
	SCE	-			
	PCD	+			

+ = significant difference; - = no significant difference; BMCR = binucleated micronucleated cell rate; Cl + MN = monocentromeric centromere-positive micronuclei; PCD = premature centromere division.
* Measured as DNA repair ability.
**10th and 90th percentiles.
*** Significantly lower values than controls.

Costa *et al.* (29) studied lymphocytes from 30 workers in pathology and anatomy laboratories and 30 matched controls. Average values for all studied parameters were significantly higher in exposed subjects than in controls (micronuclei frequency p 0.003, SCE $p<0.05$, comet tail assay (TL) $p<0.05$). There were also positive correlations between exposure levels and micronuclei frequency (p 0.001) and the results of the comet-tail assay (TL, p 0.005), but there were no significant correlations between the studied biomarkers and duration of exposure. The 8-hour time-weighted average exposure was calculated to be 0.44 ppm (range 0.04 – 1.58 ppm). During tasks with high formaldehyde exposure the average air levels were 1.50 to 4.43 ppm.

Ye *et al.* (146) found that SCE frequencies in lymphocytes of 18 factory workers exposed to formaldehyde were significantly higher than in 23 controls (average age of workers higher than controls). The reported 8-hour average for exposure was 0.8 ppm (1 mg/m^3); top exposure was 1.4 ppm (1.7 mg/m^3) (Table 1). Other Chinese authors (149) report in an abstract significant ($p<0.05$) and dose-dependent increases of DNA damage (comet assay) and chromosome damage (micronuclei test) in peripheral lymphocytes of workers exposed to formaldehyde, when compared to controls. Average air concentrations were reported to be 0.08 – 6.3 ppm (0.1 – 7.9 mg/m^3).

Jiang *et al.* (63) analyzed the occurrence of chromosome damage (CBMN assay) and DNA damage (comet tail assay; olive tail moment) in peripheral lymphocytes and the effects of polymorphism in three glutathione-S-transferase genes in 151 Chinese workers exposed to formaldehyde in two plywood factories and 112 controls not occupationally exposed to formaldehyde. The formaldehyde concentration in the breathing zone was measured for 43 of the subjects, and the 8-hour average for formaldehyde exposure in the plywood factories was reported to be 0.83 ppm (range 0.08 – 6.30 ppm). On the basis of the exposure measurements and job titles the factory workers were divided into 4 subgroups, with average (8-hour) exposures of 0.11 ppm (n=60), 0.28 ppm (n=35), 0.39 ppm (n=43) and 2.56 ppm (n=13); 0.008 ppm (the detection limit) was used to represent exposure for the control group (n=112). Both higher frequencies of micronuclei and higher values in the comet tail assay were seen when the exposed group was compared to controls. Both parameters (geometric mean/group) also showed dose-dependent increases ($p<0.001$) with increasing formaldehyde level. Significant increases in micronuclei frequency were seen in groups with average exposures $\geq$0.28 ppm, and significantly more DNA damage (comet tail assay) was observed in all exposed groups ($\geq$0.11 ppm). Division into 3 subgroups according to duration of exposure (0.6 – 1 year, reference group; 1 – 3 years; 3 – 25 years) also showed correlations to exposure for both parameters. The results also indicated that polymorphism in GST genes may modify the genotoxic effect of formaldehyde exposure, but this needs to be confirmed in larger studies.

In a new study (62), apoptosis and genotoxic effects were examined in peripheral lymphocytes from personnel in 4 pathology departments. The subjects were 37 women exposed to formaldehyde, and 21 of them formed a group that

was exposed almost exclusively to formaldehyde at work (the other 16 were exposed to solvents as well). There were also 37 controls (with a somewhat lower proportion of smokers). Exposure data (stationary monitors) were available for 3 departments, and exposure in the fourth department was assumed to be about the same. Levels of formaldehyde were in the range 0.23 – 1.20 mg/m^3 (0.19 – 0.97 ppm), 0.63 – 1.10 mg/m^3 (0.51 – 0.89 ppm) and 0.40 – 1.21 mg/m^3 (0.32 – 0.98 ppm). The 8-hour time-weighted average was 0.9 mg/m^3 (0.73 ppm), and it is reported that all the exposed subjects complained of eye irritation. The average frequency of apoptotic cells in peripheral lymphocytes was significantly higher in both exposed groups than in controls. The mutation frequency in peripheral lymphocytes (HPRT gene mutations) was significantly lower in subjects exposed mostly to formaldehyde than in controls. Cytogenetic examination showed significant increases of chromosome aberrations, mostly chromatid breaks, in both exposed groups. Significant increases in gaps and significant reductions of aneuploidy were also observed in both exposed groups. A significant increase in peripheral lymphocytes with disturbance of mitosis (premature centromere division) was also reported in both exposed groups. A significant increase of SCE was noted for a subgroup of older subjects exposed mostly to formaldehyde when compared to a younger group with the same exposure, but according to the authors this was probably due to differences in smoking habits.

Another newly published study (151) reports elevated frequencies of cyto-genetic effects characteristic of diseases such as myeloid leukemia in blood cells from Chinese factory workers exposed to formaldehyde. Two factories where formaldehyde-melamine resin was produced or used, and without exposure to other substances that are known or suspected to affect blood profile or bone marrow (e.g. benzene) and 3 control workplaces were studied. The subjects were 43 workers with formaldehyde exposure who had held the same jobs for at least 3 months, and 51 matched controls. The aneuploidy study was made with blood from a subgroup of the 10 most highly exposed workers and 12 matched controls. Numerical chromosome changes regarded as specific to myeloid leukemia and myelodysplastic syndrome (monosomy 7, trisomy 8) were quantified in peripheral myeloid stem cells: the exposed group had significantly higher frequencies of chromosome 7 monosomy (p 0.0039) and chromosome 8 trisomy (p 0.04) in metaphase than controls. The median exposure (8-hour time-weighted average) for the highly exposed subgroup was 2.14 ppm (10th percentile 1.38 ppm, 90th percentile 4.14 ppm). Different examinations of blood from all the exposed subjects showed lower proliferative potential in myeloid stem cells and lower levels of various myeloid cell types than in controls (see under Toxic effects).

The occurrence of a specific formaldehyde-DNA adduct (N^6-HOMe-dAdo) in human leukocytes is reported in a newly published study. The adduct was seen in many more smokers than nonsmokers, and the levels were significantly higher in smokers ($p<0.001$). Tobacco smoke contains formaldehyde, but it has not been established that this was the source of the formaldehyde in the adducts. According

to the authors, the results of this study may indicate that formaldehyde is a cause of tobacco-related cancer (136).

It was calculated from a pharmacokinetic model that formaldehyde should generate fewer DNA-protein crosslinks in nasal mucosa of people than in monkeys and (especially) rats (58). These assumptions have since been questioned by other researchers (119).

Carcinogenicity

Animal data

Increased cell division accompanied by cytotoxicity and cell death has been demonstrated in animal studies with inhalation exposure to formaldehyde. At lower exposure levels, however, the cell proliferation is temporary despite continued exposure. A 10- to 20-fold increase in cell replication was seen in respiratory epithelium of rats after exposure to 6 or 15 ppm formaldehyde 6 hours/day for 3 days, and in mice at the higher exposure, but no increase in cell proliferation was seen in the rats at 0.5 or 2 ppm or in the mice at 0.5, 2 or 6 ppm (66, 124). A small, temporary increase in cell proliferation was seen in rats after 1 day of exposure to 0.5 or 2 ppm, but there was no dose-response relationship (7). Zwart *et al.* (153) reported a nearly log-linear correlation between air levels and cell proliferation in nasal epithelium (region III) of rats after 3 days of exposure (6 hours/day) to 0.3, 1 or 3 ppm (significant ≥1 ppm), but after 13 weeks of exposure the average cell proliferation was no longer elevated in any exposure group (although there were concentration-dependent increases in differences between individuals). In an area nearer the front of the nose (region II), cell proliferation was low at 0.3 and 1 ppm, but at 3 ppm significantly higher cell replacement was seen after 3 days, and in this case there was little adaptation after 13 weeks. The IARC reports that a statistically significant increase in cell proliferation at 1 ppm could not be confirmed in subsequent studies, and states in its summary that neither increased cell proliferation nor elevated DNA synthesis has been shown in nasal mucosa of experimental animals subchronically exposed to formaldehyde at air levels ≤2 ppm, although a slight increase in cell replacement in specific parts of the nasal mucosa has been seen in rats at 3 ppm. Some data are reported to indicate that cytotoxicity may have a J-shaped concentration-response curve, with significantly lower cell proliferation rates in nasal epithelium at concentrations of 0.7 – 2 ppm (rats) (58). Elevated cell proliferation has been observed in the noses of rats in several studies with formaldehyde exposure of 6 ppm for a few weeks or months (95).

The TNO (Netherlands Organization for Applied Research) survey by Arts *et al.* (7) reports that elevated incidences of nasal carcinoma, as well as clear cytotoxic effects, have been observed in animal studies at air levels ≥10 ppm. Monticello *et al.* (95) showed that formaldehyde induces concentration-dependent, nonlinear increases in both cell proliferation and squamous cell carcinoma in nasal epithelium of rats. The rats were exposed to 0.7, 2, 6, 10 or 15 ppm, 6 hours/day,

5 days/week for 2 years, and the tumors could be correlated to cell proliferation in different regions of the nasal epithelium (taking into consideration the numbers of epithelial cells in these regions). Persistent and significantly elevated cell proliferation was seen at 10 and 15 ppm, and factoring in the numbers of epithelial cells in the different regions of the nose also yielded a small increase at 6 ppm. The incidences of squamous cell carcinoma were 1.1% at 6 ppm, 22% at 10 ppm and 47% at 15 ppm. The no-observed-effect concentration (NOEC) was 2 ppm. There were also low incidences of other neoplastic changes in respiratory passages, confined mostly to the nose, where they were seen only after exposure to 10 and 15 ppm; the changes included polyp-like adenomas (benign proliferative damage) with incidences of 5.6% and 9.5% respectively. The role of formaldehyde-induced chronic cell proliferation in the development of tumors in nasal mucosa was indicated in an older study with rats (145). In this study, there was an elevated incidence of squamous cell carcinoma (26%) in the noses of rats after exposure to 9.8 ppm, but only in the group with intentionally damaged (by electrocoagulation) mucous membranes and after 28 months of exposure. No elevation in tumor incidence was seen in the noses of animals with undamaged nasal mucosa at this exposure (9.8 ppm, 28 months), or in damaged or undamaged animals with 3 months of exposure to 9.2 ppm and 25 months of observation, or in any group exposed to 0.1 or 1 ppm (145).

In the study by Kerns *et al.* (66), rats and mice were exposed to 2, 5.6 or 14.3 ppm formaldehyde 6 hours/day, 5 days/week for 2 years. Squamous cell carcinomas were observed in the nasal cavities of 44% of the rats at 14.3 ppm and 0.9% (not significant) at 5.6 ppm. A few polyp-like adenomas in nasal cavities were observed at all exposures, with a significant ($p<0.05$) trend for male rats (0.8% at 0 ppm, 3.4% at 2 ppm, 5% at 5.6 ppm, 3.4% at 14.3 ppm). In the mice, there were squamous cell carcinomas in 0.8% at 14.3 ppm (not significant). In a much smaller study in which rats were exposed to 0.3, 2 or 15 ppm formaldehyde 6 hours/day, 5 days/week for 28 months, squamous cell carcinomas were reported in the nasal cavities of 41% at 15 ppm but were not seen at the lower exposures (65).

An incompletely described study, in which rats were given formaldehyde in drinking water (10, 50, 100, 500, 1,000 or 1,500 mg/l, stabilized with 0.3% methanol), methanol in drinking water (15 mg/l), or plain drinking water for 2 years, reports significant increases in total numbers of malignant tumors and hemolymphoreticular tumors in the groups exposed to formaldehyde (116). In more recent calculations by the IARC, using the methanol groups (both sexes) as controls, the total number of animals with malignant tumors was still significant ($p<0.01$) for male rats in the highest exposure group and for interstitial cell adenomas in testes ($p<0.01$) of males in the second-highest exposure group. The elevations in incidences of lymphoma and leukemia were also statistically significant ($p<0.01$) for males in the highest exposure group, and there was a significant dose-response correlation for the incidence of hemolymphoreticular tumors in the males ($p<0.01$). The IARC (58) points out that the results for

lymphoma and leukemia were treated together, incidence data for historic controls were not reported, no treatment-related non-oncological pathological changes were observed in the histopathological examinations, and that it is not clear how many animals had the hemolymphoreticular tumors. The study could therefore not be assessed. In several other studies, no increases of lymphatic or bone-marrow related cancers were seen in exposed animals, and the IARC has observed that there is no evidence that long-term exposure to formaldehyde causes leukemia in laboratory rodents (58).

The IARC nevertheless states that there is "sufficient evidence" that formaldehyde is carcinogenic to laboratory animals. The IARC summary states that formaldehyde causes tumors in the nasal cavities of rats and that the data indicate that both genotoxicity and cytotoxicity play major roles in development of these cancers. Cell proliferation increases considerably at air levels around 6 ppm, and the increased cell proliferation seems to greatly enhance the genotoxic effect of formaldehyde. At concentrations above this level, clear increases of malignancies have been observed in the noses of rats (58).

Human data

The IARC (58) reports that correlations between formaldehyde and cancer have been examined in more than 25 cohort studies. There are also numerous case-control studies and some meta-analyses. The most important studies cited by the IARC, as well as some newer studies, are reviewed below. The IARC, in its summary judgement, placed formaldehyde in Group 1: "carcinogenic to humans" (8, 58, 59).

Nasopharyngeal cancer

Elevated mortality due to cancer in the upper (nasopharyngeal) part of the throat, compared to the entire population, is reported in a follow-up (until 1994) of an NCI-led study. The cohort comprised over 25,000 employees at 10 factories in the USA where formaldehyde was produced or used. The SMR, based on 8 exposed cases, was 2.10 (95% CI 1.05 – 4.21; exact 95% CI 0.91 – 4.14); one of the exposed cases, however, was misclassified (oropharyngeal cancer). Most of the exposed cases (5, sometimes counted as 6 cases) were at the same company (cluster). Two unexposed persons in the cohort also died of nasopharyngeal cancer. The median exposures for the formaldehyde-exposed workers were 0.3 ppm (range 0 – 4.25 ppm) for average exposure level and 0.6 ppm-years (range 0 – 107.4 ppm-years) for cumulative exposure. The relative risk (RR) of death due to nasopharyngeal cancer (with the misclassified case excluded from the calculation) was 1.83 (not significant) for the group with the highest top exposures (≥4 ppm), and all 7 of the exposed cases were in this category. The RR was 1.67 (not significant) for the highest average exposure (≥1 ppm), 4.14 for the highest cumulative exposure (≥5.5 ppm-years), and 4.18 for the longest duration of exposure (≥15 years). There were statistically significant trends for top exposure ($p<0.001$) and cumulative exposure ($p<0.025$) (46).

Other authors, after re-analyzing the NCI cohort, analyzing the company with most of the cases of nasopharyngeal cancer, and updating the data, report results that give little support to the hypothesis of a causative correlation between formaldehyde exposure and elevated risk of death due to nasopharyngeal cancer. Among the possible reasons given by these authors for the high incidence of nasopharyngeal cancer at the factory with most of the cases in the 2004 study by Hauptmann *et al.* (46) was prior employment in the metal industry, with exposure to sulfuric acid mist, heat etc. (9, 15, 34, 86, 88, 89, 90). (The Criteria Group notes that none of the mentioned exposures is known to cause nasopharyngeal cancer). The IARC (58), however, notes that exposure estimates for the factory in question were about 10 times higher in the Hauptmann study (46) and were based largely on occupational hygiene estimates, whereas exposure estimates in the studies by Marsh and others were based only on information supplied by the company (see Reference 58). The median values for exposure levels in this company were reported to be 1.023 ppm and 0.14 ppm respectively (34).

In a cohort of about 11,000 workers at three garment manufacturers in the USA, followed by NIOSH through 1998, there were no deaths due to nasopharyngeal cancer (0.96 expected). It is difficult, however, to ascertain an increase in a rare form of cancer (low power), and individual exposure estimates are also lacking. The average formaldehyde levels in various departments were in the range 0.09 – 0.20 ppm in the early 1980s, but may have previously been much higher (58, 105). In a British cohort study (with updates through 2000) of about 14,000 workers at 6 factories where formaldehyde was produced or used, there was 1 death (vs. 2 expected) due to nasopharyngeal cancer (26). Division into exposure groups with time-weighted average exposures of <0.1 ppm (background exposure), 0.1 – 0.5 ppm (low exposure), 0.6 – 2 ppm (medium exposure) and >2 ppm (high exposure) was based mostly on measurements made after 1970, and an estimated 28% of the workers in the cohort had an average exposure ≥2 ppm (26, 34). In a study based on the Danish cancer register, cancer incidence in 1970 – 1984 was examined for 2,041 Danish men employed since 1964 (at least 10 years prior to diagnosis) in 265 companies with identified exposure to formaldehyde. There were 4 observed (vs. 3.2 expected) cases of nasopharyngeal cancer (standardized proportional incidence ratio (SPIR) 1.3, 95% CI 0.3 – 3.2). This study contains no direct measures of formaldehyde exposure (43).

A proportional mortality ratio (PMR) of 2.16 (95% CI 0.59 – 5.54) for nasopharyngeal cancer, based on 4 observed cases, is also reported in an analysis of data on about 4,000 men in the undertaking business, including embalmers. The total mortality pattern in this particular group may differ from that of general population, however. There are no exposure estimates in this study (48). In a case-control study with a cohort composed of dead people who had been in the undertaking business (deaths 1960 – 1985), there were 4 cases of nasopharyngeal cancer, but only 2 of these had worked with embalming (OR 0.1, 95% CI 0.01 – 1.2) (47).

Several other case-control studies have been published, and some of them have indicated correlations between formaldehyde exposure and nasopharyngeal cancer, but exposure estimates in this type of study are retrospective and misclassification may occur (58). No correlation between occupational exposure to formaldehyde and nasopharyngeal cancer was observed in a study from Malaysia, with 282 cases of nasopharyngeal cancer (squamous cell carcinoma) (53% of the cases diagnosed during the period) and matched controls although exposures to wood dust and heat were associated with nasopharyngeal cancer. Only 9.9% of the cases and 8.2% of the controls were reported to have been exposed to formaldehyde, however (6). In a Chinese case-control study with 375 cases (>90% unkeratinized and undifferentiated carcinomas) and 325 matched controls, occupational exposures to substances including wood dust and formaldehyde were mapped, and a slight (not significant) elevation in risk of nasopharyngeal carcinoma (RR 1.4, 95% CI 0.93 – 2.2) was seen for "individuals ever exposed to formaldehyde" (74 cases, 41 controls). A stronger correlation was seen when the analysis was limited to individuals who were seropositive for the Epstein-Barr virus (EBV) (360 cases, 94 controls; RR 2.7, 95% CI 1.2 – 6.2). No clear dose-response patterns were seen for either duration of exposure or cumulative exposure to formaldehyde, although stronger effects were seen with analysis of individuals with high average intensity or probability of exposure. For example, the estimated RRs for ≤ and >10 years of exposure in the group with high exposure intensity were 2.1 (95% CI 0.94 – 4.8) and 2.1 (95% CI 1.0 – 4.2). The authors concluded that occupational exposure to wood dust probably contributes to the development of nasopharyngeal cancer and that the correlation to formaldehyde exposure is less clear (55). The same research group, in a case-control study from the Philippines (104 cases, 205 controls), reported an elevated risk of nasopharyngeal carcinoma for persons who had first been exposed to formaldehyde ≥25 years prior to diagnosis (adjusted RR 4.0, 95% CI 1.3 – 12.3; 14 cases). EBV antibodies were not determined in this study (140).

In a case-control study from the USA (196 cases, 244 controls), focused on cases of epithelial cell carcinoma in the nasopharyngeal region, occupational exposure to formaldehyde (but hardly exposure to wood dust) was associated to an elevated risk: 40.3% of the cases and 32.4% of the controls were potentially exposed to formaldehyde. The correlations applied only to certain subtypes of nasopharyngeal carcinoma (epithelial carcinomas). The ORs for persons "ever" exposed, with likelihoods classified as possible/probable/definite, probable/definite, or definite, were 1.6 (95% CI 1.0 – 2.8), 2.1 (95% CI 1.1 – 4.2), and 13.3 (95% CI 2.5 – 70) respectively. There were significant risk trends for greater duration of exposure (p 0.014) and cumulative exposure (p 0.033). The OR for cumulative exposure >1.10 ppm-years with possible/probable/definite exposure to formaldehyde was 3.0 (95% CI 1.3 – 6.6), compared to unexposed persons, and the corresponding OR for exposure duration >18 years was 2.7 (95% CI 1.2 – 6.0). The risk trends were stronger ($p<0.001$) when only jobs with "definite" exposure were used in the calculations (few persons), but weaker when only jobs with "probable/definite" exposure were used (133).

Bosetti *et al.* (15) made a meta-analysis using cohort studies published prior to March, 2007 and containing information on formaldehyde exposure and cancer risk (case-control studies were regarded as providing less accurate exposure information and were therefore not used). For 3 cohort studies of industrial workers exposed to formaldehyde (26, 46, 105), with a total of 9 observed (vs. 6.8 expected) cases, the SMR was 1.33 (95% CI 0.61 – 2.53). When the cluster of 6 deaths at the same factory was excluded, the total RR dropped to 0.49 (3 deaths). When two other studies that reported observed but not expected deaths due to nasopharyngeal cancer were also included, the SMR was 1.40 (95% CI 0.67 – 2.57) (15). In a new meta-analysis based on 7 cohort studies (the factory with the cluster was not included), the calculated RR was 0.72 (95% CI 0.40 – 1.29). Including all ten of the factories in the study by Hauptmann *et al.* (46) yielded a combined risk estimate of 1.17 (95% CI 0.73 – 1.86). The OR based on 6 case-control studies was 1.22 (95% CI 1.00 – 1.50). If only the 4 case-control studies adjusted for smoking were used in the calculation, the OR was 1.10 (95% CI 0.80 – 1.51) (9).

In the assessment of the IARC, the epidemiological data provide "sufficient evidence" that formaldehyde causes nasopharyngeal cancer. This judgement is based primarily on data from the study by Hauptmann *et al.* (2004) (46), but is also supported by other epidemiological data (58, 59). The IARC judgement supporting a correlation between occupational exposure to formaldehyde and nasopharyngeal cancer, with the consequent carcinogenicity classification, has been questioned in some studies (9, 34).

Sinonasal cancer

No significant increase in risk of death due to cancers in the nose and nasal cavity (SMR 1.19, 95% CI 0.38 – 3.68; 3 cases) was seen for workers exposed to formaldehyde in the large cohort study of workers at 10 factories in the USA where formaldehyde was produced or used (46). In the British cohort study of workers at 6 factories with production or use of formaldehyde there were 2 deaths due to sinonasal cancer vs. 2.3 expected (26). No deaths due to nasal cancer (0.16 expected) are reported in the cohort study of workers at 3 garment manufacturers in the USA (105). The study based on about 2,000 men in the Danish cancer register, however, showed an elevated risk for sinonasal cancer, based on 13 reported cases (SPIR 2.3, 95% CI 1.3 – 4.0). Stratification showed an elevated risk, based on 9 reported cases (5 of them described as squamous cell carcinomas) in subjects "moderately" exposed to formaldehyde, but probably not to wood dust (SPIR 3.0, 95% CI 1.4 – 5.7). This study contains no direct measures of exposure (43). No deaths due to sinonasal cancer (vs. 1.7 expected) are reported in an analysis of data on about 4,000 men in the undertaking business, including embalmers (48).

Luce *et al.* (83) published a pooled analysis of data on sinonasal cancer (432 cases of squamous cell carcinoma and 195 cases of adenocarcinoma) from 12 case-control studies, using a job-exposure matrix for estimates of exposure

intensity and probability of exposure. A correlation between increasing cumulative formaldehyde exposure and increasing risk of adenocarcinoma was observed for the men (128 cases): the ORs for low, medium and high cumulative exposure were 0.7 (95% CI 0.3 – 1.9), 2.4 (95% CI 1.3 – 4.5) and 3.0 (95% CI 1.5 – 5.7). For women with the highest cumulative exposure, the OR was 6.2 (95% CI 2.0 – 19.7; 5 cases). Nearly all the formaldehyde-exposed men were also exposed to wood dust, however. Only 18 cases (11 men, 7 women) were categorized as never exposed to wood dust. For men with little or no exposure to wood dust and high cumulative exposure to formaldehyde (5 cases) the OR for adenocarcinoma was 2.2 (95% CI 0.8 – 6.3). The ORs for squamous cell carcinoma with low, medium and high cumulative exposure were given in the study as 1.2 (95% CI 0.8 – 1.8), 1.1 (95% CI 0.8 – 1.6), and 1.2 (95% CI 0.8 – 1.8) for men, and 0.6 (95% CI 0.2 – 1.4), 1.3 (95% CI 0.6 – 3.2) and 1.5 (95% CI 0.6 – 3.8) for women.

The IARC states that there is "limited evidence" of a correlation between formaldehyde exposure and sinonasal cancer in humans (58, 59).

Lymphatic and myeloid cancer

A cohort study made by the National Cancer Institute in the USA reported mortality rates for lymphatic and myeloid cancers (SMR 0.80, 95% CI 0.69 – 0.94) and leukemia (SMR 0.85, 95% CI 0.67 – 1.09) that were lower for workers exposed to formaldehyde than for the entire population. The study covered about 25,000 employees at factories producing or using formaldehyde (the NCI cohort). Internal comparisons, however, revealed significant trends for higher risk related to top exposures, notably for lymphatic and myeloid cancers (p for trend <0.002), leukemia (p for trend <0.004) and myeloid leukemia (p for trend 0.009). In the group with top exposures of 2 – 3.9 ppm the RR for myeloid leukemia was 2.43 (95% CI 0.81 –7.25) and for top exposures ≥4 ppm the RR was 3.46 (95% CI 1.27 – 9.43) compared to the reference category (0.1 – 1.9 ppm). For average exposures of 0.5 – 0.9 ppm the RR for myeloid leukemia was 1.15 (95% CI 0.41 – 3.23), and for average exposures ≥1 ppm the RR was 2.49 (95% CI 1.03 – 6.03) (p for trend 0.09), compared to the reference category (0.1 – 0.4 ppm). Neither cumulative exposure nor duration of exposure showed a statistical correlation to risk for myeloid leukemia (45). A thorough re-analysis of the NCI data and some further analyses, however, provided less support for the causal connection between formaldehyde exposure and deaths due to leukemia and myeloid leukemia indicated in the NCI study. One point mentioned was that the elevated RRs for all leukemia/myeloid leukemia related to top exposures were due to significantly reduced mortality in the reference category. The revised SMRs for myeloid leukemia in the groups with medium and high top exposures were calculated to be 0.94 (95% CI 0.41 – 1.85) and 1.42 (95% CI 0.78 – 2.38), in comparison to the population as a whole. Further, the SMR for myeloid leukemia was given as 1.45 (95% CI 0.66 – 2.75) for average exposures ≥1 ppm, whereas division into tertiles yielded an SMR of 1.02 (95% CI 0.47 – 1.94) for the highest tertile (≥0.74 ppm) in comparison to the general population (15, 87).

In a newly published (36) follow-up (through 2004) of the NCI cohort, it was observed in general that 1,006 deaths in the 1980 – 1994 period had not been included in the earlier analysis, and that some cases were misclassified. New calculations yielded a somewhat smaller elevation in risk for myeloid leukemia. The RR for the highest top exposure category (≥4 ppm) was reported to be 2.79 (95% CI 1.08 – 7.21; p for trend 0.02) compared to the reference category, and the corresponding RR for the group with average exposure (intensity) was given as 2.19 (95% CI 0.92 – 5.25, p for trend 0.11). No elevation in risk for myeloid leukemia in the group with highest top exposure was seen for the 1995 – 2004 period (RR 0.71), and calculations based on the entire study period yielded an RR of 1.78 (95% CI 0.87 – 3.64) for this group. It was also reported that, for the entire study period, the RRs for Hodgkin's disease were 3.30 (95% CI 1.04 – 10.50) for top exposures of 2 – 3.9 ppm, 3.96 (95% CI 1.31 – 12.02, p for trend 0.01) for top exposures ≥4 ppm, and 3.62 (95% CI 1.41 – 9.31) for the group with average exposure (intensity) of 0.5 – 0.9 ppm, compared to the reference category. No other significant correlations were seen between average exposure or cumulative exposure to formaldehyde and lymphatic or myeloid cancers. The authors regard the correlation between formaldehyde and Hodgkin's disease as very hard to explain (36).

In a cohort of about 11,000 workers in the garment industry (the NIOSH cohort), the SMRs were 1.09 (95% CI 0.70 – 1.63) for leukemia and 1.44 (95% CI 0.80 – 2.37) for myeloid leukemia, compared to the population as a whole. Mortality due to myeloid leukemia was significantly elevated among workers with 20 or more years since initial exposure (13/15 cases, SMR 1.91), but the trend was not significant. For workers with both ≥10 years of exposure and ≥20 years since initial exposure, the SMRs were 1.92 (95% CI 1.08 – 3.17; 15 deaths) for leukemia and 2.55 (95% CI 1.10 – 5.03; 8 deaths) for myeloid leukemia (analysis for multiple causes of death). Average exposures during the early 1980s were in the range 0.09 – 0.20 ppm, but may previously have been much higher (58, 105). Pinkerton *et al.* (105) observe that although the results of this study are not conclusive they support a possible correlation between formaldehyde exposure and death from myeloid leukemia. No elevation in leukemia risk was seen in a British cohort study of 14,000 workers in factories where formaldehyde was produced or used: the SMR was 0.91 (95% CI 0.62 – 1.29) (26). In the study based on the Danish cancer register, covering about 2,000 Danish workers at companies with exposure to formaldehyde, the SMR for leukemia was 0.8 (95% CI 0.6 – 1.6) (43). The specific risk for myeloid leukemia was not calculated in these two studies.

In an analysis of data on about 4,000 men in the undertaking business (funeral directors and embalmers), the PMR for myeloid leukemia was 1.57 (95% CI 1.01 – 2.34; 24 reported deaths). The mortality pattern in this particular group of people may differ from that of the general population; moreover, this study contains no exposure estimates (48). Hauptmann *et al.* (47) reported correlations between myeloid leukemia and formaldehyde exposure in a case-control study of causes

of death among a cohort comprised of people who had worked in the undertaking business (deaths in the 1960 – 1985 period). The study included 168 cases of lymphatic and myeloid cancers (34 were myeloid leukemia), 48 cases of brain tumors, 4 cases of nasopharyngeal cancer, and 265 matched controls (other causes of death). Their jobs were described on the individual level via information on number of embalmings etc., and formaldehyde exposure (cumulative exposure, average exposure, top exposure) was estimated with mathematical models. For persons who had worked with embalming (group averages, cases and controls) the calculated 8-hour time-weighted average for air levels of formaldehyde was 0.1 – 0.2 ppm, and average air levels during an embalming were in the range 1.5 – 1.8 ppm. Calculated top exposures were 8.1 – 10.5 ppm. The OR for myeloid leukemia for persons who had at some time done embalming (n=33) vs. never done embalming (n=1, reference) was 11.2 (95% CI 1.3 – 95.6; p 0.027). A significant trend (p 0.02) for myeloid leukemia with increasing number of years doing embalming was reported: the ORs were 5.0 (95% CI 0.5 – 51.6) for >0 – 20, 12.9 (95% CI 1.4 – 117.1) for >20 – 34, and 13.6 (95% CI 1.6 – 119.7) for >34. No significant trend was seen for number of embalmings (significant ORs with wide and overlapping confidence intervals). Nor were there significant trends for cumulative exposure or for various measures of average exposure (significant ORs with wide and overlapping confidence intervals). A significant trend (p 0.036) was noted for myeloid leukemia and top exposures, however. The ORs were 15.2 (95% CI 1.6 – 141.6) for >0 – 7.0 ppm, 8.0 (95% CI 0.9 – 74.0) for >7.0 – 9.3 ppm, and 13.0 (95% CI 1.4 – 116.9) for >9.3 ppm, compared to the reference (0 ppm, 1 case). Analyses of data for myeloid leukemia using an expanded reference group (<500 embalmings; 5 cases) resulted in generally lower and fewer significant ORs, but significant trends as above. Analyses using the various parameters for formaldehyde exposure/jobs in the study revealed no correlations to deaths due to other types of lymphatic or myeloid tumor diseases (e.g. non-Hodgkin's lymphoma, multiple myeloma), and the results for brain tumors were considered inconclusive.

Bosetti *et al.* (15), on combining the results in 4 different cohort studies of industrial workers (including studies 26, 45, 105), reported an estimated RR of 0.90 (95% CI 0.75 – 1.07; 122 cases) for leukemias, and a pooled group of pathologists, embalmers etc. from 8 cohorts yielded an estimated RR of 1.39 (95% CI 1.15 – 1.68; 106 cases) for leukemias. Both SMR and PMR were calculated. For lymphatic and myeloid cancers, this procedure yielded RRs of 0.85 (95% CI 0.74 – 0.96; 234 cases) for the first group and 1.31 (95% CI 1.16 – 1.48; 263 cases) for the second. Collins and Lineker (28) calculated relative risks for leukemia in a meta-analysis based on the results of 18 epidemiological studies (cohort, PMR, case-control) published in 1975 – 2004. The RR for leukemia, based on a total of 287 cases in all studies, was calculated to be 1.1 (95% CI 1.0 – 1.2). Elevated relative risks were seen in studies of embalmers (RR 1.6, 95% CI 1.2 – 2.0, 78 cases) and pathologists/anatomists (RR 1.4, 95% CI 1.0 – 1.9; 45 cases), but not among industrial workers (RR 0.9, 95% CI 0.8 – 1.0, 164 cases)

(28). In a new meta-analysis (150) focused on the groups with highest formaldehyde exposure in each of 15 studies, the calculated RR for leukemia was 1.54 (95% CI 1.18– 2.00, p<0.001). The highest relative risk (RR 1.90, 95% CI 1.31 – 2.76; p 0.001) was noted for myeloid leukemia, based on 6 studies: 2 were cohorts of industrial workers (45, 105) and 4 were studies of embalmers and anatomists. Results for leukemia and myeloid leukemia remain positive (although the RRs are somewhat reduced) if the study by Freeman *et al.* (36) is used in the meta-analysis instead of the study by Hauptmann *et al.* (45) (L Zhang, personal communication with J Högberg). Some elevation in risk for multiple myeloma was also reported in the study by Zhang *et al.* (150), with an RR of 1.31 (95% CI 1.02 – 1.67). Three possible mechanisms by which formaldehyde could damage hematopoietic stem cells are also presented in this study: 1. Formaldehyde could be taken up in the blood, transported to the bone marrow and there damage the stem cells, which then develop into leukemia cells. 2. Formaldehyde could damage hematopoietic stem cells and/or progenitor cells that circulate in the blood. These damaged cells could then be incorporated into the bone marrow and develop into leukemia cells. 3. Formaldehyde could induce promutagenic/mutagenic damage in primitive pluripotent stem cells in the respiratory passages or oral cavity. These damaged cells could then be transported in blood to the bone marrow and there develop into leukemia cells (150).

The study by Zhang *et al.* (150) is criticized in a subsequently published meta-analysis (9). It is pointed out that the risk estimates in the included studies (15 on leukemia, 6 on myeloid leukemia) varied widely, with consequent problems in calculating reasonably accurate combined risk estimates, and that the definitions of "high exposure" used in the different studies also vary. Proportionality studies were not included in the meta-analysis by Bachand *et al.* (9), which gives a combined relative risk of 1.05 for leukemia (95% CI 0.93 – 1.20; 15 cohort studies) and 1.09 for myeloid leukemia (95% CI 0.84 – 1,40; 3 studies). Division according to type of work yielded an RR of 0.99 (95% CI 0.86 – 1.15, 8 studies) for industrial workers and 1.28 (95% CI 0.98 – 1.66) for pathologists, embalmers, laboratory technicians etc. (The 2009 study by Hauptmann *et al.* (47) is not included in this meta-analysis.)

The earlier IARC assessment was that there was strong but not conclusive evidence of a causal connection between leukemia and occupational exposure to formaldehyde, and it was mentioned that no mechanism for induction of myeloid leukemia in humans could be identified. In its most recent assessment, however, the IARC states that there is "sufficient evidence" of a causal connection between leukemia and formaldehyde exposure. Stronger epidemiological evidence, information on changes in blood profile indicating effects on bone marrow, and numerical chromosome aberrations in myeloid stem cells from formaldehyde-exposed workers, characteristic of such diseases as myeloid leukemia, were decisive to this new assessment (the need of confirmation by further studies was also mentioned, however) (8, 58, 59). The new data taken into consideration include the studies by Zhang *et al.* (151) (reviewed here in the sections Toxic

effects and Mutagenicity, genotoxicity) and Hauptmann *et al.* (47) (reviewed under Carcinogenicity).

Both the possibility that formaldehyde exposure can cause leukemia and the IARC assessment of human data on leukemia have been questioned in some studies (9, 51, 107).

Other types of cancer

Correlations between formaldehyde exposure and cancer in other locations, including the mouth, oropharynx (throat behind the oral cavity), hypopharynx (throat between the oral cavity and the larynx), larynx, lungs, pancreas and brain, have been reported, but according to the IARC the overall epidemiological data do not support a causal relationship to formaldehyde exposure for cancers in these locations (58).

Effects on reproduction

A survey by Collins *et al.* (27) summarizes about 10 epidemiological studies that examined effects on reproduction due to occupational exposure to formaldehyde. Simultaneous exposures to one or more substances with known toxic effects on reproduction were reported in most of the studies, and most of them were not designed specifically to assess formaldehyde exposure (27, 58). They focused mostly the occurrence of spontaneous abortions among women exposed to formaldehyde, and the results were equivocal. A meta-analysis based on 7 studies yielded an RR of 1.6 (95% CI 0.9 – 2.7); 3 of the studies were cohort studies that had a combined relative risk of 1.7 (95% CI 1.2 –2.3). One of the studies (128) reported an elevated risk of spontaneous abortion among female woodworkers exposed to formaldehyde: the ORs were 3.2 (95% CI 1.2 – 8.3) for high exposure, 1.8 (95% CI 0.8 – 4.0) for medium exposure, and 2.4 (95% CI 1.2 – 4.8) for low exposure. This study was designed to examine fertility, however (see below), and the abortion analysis was limited to 52 pregnancies. Moreover, these subjects were also exposed to organic solvents.

For other parameters examined in the studies (birth defects, birth weights, infertility) the results were also equivocal, though mostly without significant elevations in risk. The studies were few, however, and were reported to have weaknesses such as lack of control for confounding factors (27, 58). Time to desired pregnancy (TTP) was examined in the retrospective study of female woodworkers (128). There were 602 women in the analyzed group. Reduced fertility, expressed as fecundability density ratio (FDR), was shown in the group with high exposure to formaldehyde (n=39); the FDR was 0.64 (95% CI 0.43 – 0.92). The FDR was lower (0.51, 95% CI 0.28 – 0.92) for women in this group (n=17) who did not use gloves; the FDR for highly exposed women who used gloves was 0.79 (95% CI 0.47 – 1.23). However, the response rate in this study was only 64% (699/1,094). A highest measured exposure level of 1 ppm and an average exposure level of 0.33 ppm are given for the group with high

formaldehyde exposure. Exposures to phenol, dust, wood dust and organic solvents were shown to be unrelated to TTP. High levels of formaldehyde were also reported to be correlated to an elevated risk of endometriosis (OR 4.5, 95% CI 1.0 – 20.0). (Exposure to organic solvents was also correlated to endometriosis). A new Chinese survey (127) reports that heavier menstruations or irregular menstrual cycles have been seen in three studies of women occupationally exposed to formaldehyde. The exposure levels given in the two studies that reported increases in irregular menstrual cycles were about 0.6 – 4.8 ppm (0.8 – 6 mg/m^3) in one study and >0.4 ppm, in some cases 3.2 ppm (>0.5 mg/m^3, 4 mg/m^3) in the other. No further details are given, however, and since the original data are not available these studies can not be evaluated.

One study looked into the possibility of a correlation between paternal exposure to formaldehyde and spontaneous abortions, but no correlation was found (76). In another study, effects on sperm (including sperm counts, morphology) were examined in 11 pathologists and pathology assistants and 11 matched controls, but no significant differences between the groups were observed. Exposure was intermittent, with a time-weighted average of 0.6 – 1.3 ppm. Maximum formaldehyde levels (during specific tasks) were reported to be 5.8 ppm (137).

The IARC reports that effects on pregnancy and fetal development have been studied in laboratory animals exposed to formaldehyde by inhalation, but were not clearly demonstrated at doses that were not toxic to the mothers (58). Saillenfait *et al.* (110), however, reported that formaldehyde was slightly fetotoxic to rats at an air level (20 ppm) that did not cause clear indications of maternal toxicity. In this study, exposures were 0, 5, 10, 20 or 40 ppm, 6 hours/day on days 6 to 20 of gestation, and significantly lower fetal weight was noted at 20 ppm ($p<0.05$, only male fetuses) and 40 ppm. Maternal toxicity, expressed as lower weights/lower weight gain, was seen at 40 ppm. No significant differences between the groups were seen for other studied parameters (pregnancy incidence, number of implantations, prenatal death, developmental defects, sex ratio). In another study, rats were exposed to 2, 5 or 10 ppm 6 hours/day on days 6 to 15 of gestation, and a significant and concentration-dependent increase in incidence of reduced ossification (pelvic girdle) was observed in the fetuses at 5 and 10 ppm, but was associated with larger litters and lower fetal weights and not regarded as a formaldehyde-related effect. There were no other significant effects on the studied parameters (including number of corpora lutea, implantation locations, resorptions and living fetuses, fetal weights, sex ratio, developmental defects, anomalies) in any group. Feed consumption and weight gain were significantly lower ($p<0.05$) in the mothers at 10 ppm (91). In a Russian study (Kitaeva *et al.* 1990, reviewed in Reference 58) female rats were exposed to 0.4 or 1.2 ppm (0.5 or 1.5 mg/m^3) formaldehyde, 4 hours/day for up to 4 months, and then mated with untreated males. The embryos were examined 2 or 3 days after mating, and it is reported that a significant number of degenerating embryos was found in the high-dose group. Cytogenetic analysis showed that the embryos had no more chromosome aberrations than controls.

Significant and dose-dependent effects in the form of reduced serum testosterone levels, reduced diameters of seminiferous tubules (in testes) and higher synthesis of a stress marker in spermatogonia, spermatocytes and spermatids were observed in rats after inhalation exposure to 5 or 10 ppm formaldehyde 8 hours/day, 5 days/week for 3 months. The animals showed effects of severe irritation (including hemorrhages in nasal mucosa) and had reduced intakes of feed and water (155). Significantly lower testes weights, histopathological and biochemical changes in testes, and effects on sperm (larger proportion of abnormal and reduced proportion of motile sperm, lower sperm counts) were observed in another study in which rats were exposed to 8 ppm (10 mg/m^3) formaldehyde (152). An incompletely described study (39) reports that several types of changes were seen in histopathological examinations of testes from juvenile rats exposed to formaldehyde from cadavers in a room where the average concentration was 1.5 ppm (compared to unexposed controls). The animals were exposed for 18 weeks, 2 or 4 hours/day, 4 days/week or 2 hours/day, 2 days/week. Inhibited spermatogenesis was observed in the most highly exposed group (Table 3).

In a study in which male rats were given intraperitoneal injections of a 37% formalin solution with 10% methanol in doses of 0.125 – 0.5 mg/kg body weight/day for 5 days (controls were injected with water) and mated with untreated females 1 to 3 weeks after the treatment, dose-dependent effects were observed in a dominant lethal test, and fertility was also impaired (number of fertile matings, numbers of implantations and living embryos per female). The effects were observed primarily during the first 2 weeks after the treatment. When the rats were killed (3 weeks after the last injection) it was also observed that sperm counts declined with rising dose, and significant (at all doses), dose-dependent elevations in frequencies of abnormal sperm were noted in the sperm head abnormality test (101). Negative effects on sperm (number, morphology, viability, motility) have been observed in other studies in which laboratory rodents were given repeated intraperitoneal injections of formaldehyde in doses of 4 to 10 mg/kg b.w./day (Yi *et al.* 2000, cited in References 58, 84). Studies using intraperitoneal injections to assess the effects of formaldehyde on reproductive cells are of doubtful relevance for workers exposed to formaldehyde by inhalation (27, 58).

Dose-effect/dose-response relationships

When volunteers are exposed to formaldehyde under controlled conditions, eye irritation is usually the effect that appears at the lowest level in short-term exposures. In one study (1), about 20% of persons exposed to 0.24 ppm reported feeling slight eye irritation. In the study by Lang *et al.* (73), very slight subjectively estimated eye irritation was reported at 0.3 ppm. Objective indications of eye irritation such as higher blinking frequency and reddened conjunctiva were seen at 0.5 ppm with peaks of 1 ppm, but not at 0.5 ppm without exposure peaks (73). Nasal symptoms (sneezing, itching, swollen mucous membranes) and objective indications of a general inflammation process in nasal mucosa were

reported in subjects exposed to an average 0.4 ppm (0.2 – 0.6 ppm) (70). Another respiratory effect of formaldehyde is increased sensitivity to other substances. In one study (24), asthmatics allergic to mite allergen showed heightened sensitivity to the mite allergen after 30 minutes of exposure to 0.07 ppm formaldehyde (Table 2).

Many studies of persons occupationally exposed to formaldehyde have been published, but exposures in these studies are more uncertain. The IARC (58) states in its summary that there is evidence that formaldehyde is genotoxic to humans (and experimental animals – see below). In the study by Ballarin *et al.* (10) elevated frequencies of micronucleated cells were observed in nasal mucosa of workers exposed to formaldehyde in a plywood factory. Levels of formaldehyde were 0.07 – 0.3 ppm (8-hour averages) (10, 58). The IARC points out, however, that wood dust may have contributed to the elevated incidence of micronucleated cells in the exposed group. The studies by Ying *et al.* report significantly higher average frequencies of micronucleated cells in both nasal and oral mucosa of anatomy students intermittently exposed to formaldehyde. On the other hand, no significant increase of micronuclei or SCE was seen in peripheral lymphocytes. Time-weighted average exposure during the 3-hour anatomy lessons was 0.4 ppm (range 0.06 – 1.04 ppm) (58, 147, 148). According to the IARC, however, SCE and DPC in peripheral lymphocytes showed a correlation to formaldehyde exposure (in pathology departments) in the studies by Shaham *et al.* (114, 115), although it was mentioned that these endpoints are of doubtful relevance to assessing the mutagenicity or carcinogenicity of formaldehyde (58). The average air level (15-minute exposure measurements) for the low-exposure groups in these two studies was 0.4 ppm.

Some of the more recent studies have reported elevated occurrences of micronuclei in lymphocytes of laboratory technicians in pathology and anatomy laboratories (29, 60, 102). Reported average exposure levels (8 hours) were 0.1 – 0.44 ppm, although considerably higher exposures were reported to occur during brief periods. A study (63) of workers exposed to formaldehyde in plywood factories also reports elevated occurrences of micronuclei in lymphocytes, as well as higher values in a test of DNA damage. The increases were dose-related, with significant increases in groups with average 8-hour exposures of ≥0.28 ppm (micronuclei) and ≥0.11 ppm (DNA damage). Significant increases in apoptosis and in chromosome aberrations in peripheral lymphocytes are reported in a study of personnel in pathology departments (62). Air levels were reported to be in the range 0.19 – 0.98 ppm, with a time-weighted 8-hour average of 0.73 ppm (Table 1). Effects on blood profile, with lower total numbers of white blood cells and lower levels of lymphocytes, granulocytes, thrombocytes and red blood cells, as well as negative effects on the proliferation potential of cultured myeloid progenitor cells, were observed in a group of Chinese workers with relatively high average exposure to formaldehyde (151). The median exposure level for the group (8-hour exposure) was 1.28 ppm. In a subgroup with high exposure (median 8-hour exposure 2.14 ppm), there was a reported increase of numerical chromosome

changes (monosomy 7, trisomy 8) typical of myeloid leukemia and myelodysplastic syndrome (Table 1). The fact that several studies have shown genotoxic effects in peripheral cells of humans, whereas corresponding studies with animals have usually been negative, can be interpreted as an effect of species differences (humans may be more sensitive than experimental animals), but it may also be explained by the fact that the humans were often exposed to exposure peaks and/or other substances. Furthermore, there are no relevant animal models for myeloid leukemia (58).

In a large American cohort of industrial workers (the NCI cohort) there were significant trends for elevated risk related to top exposures to formaldehyde, especially for myeloid leukemia (45). The RRs for myeloid leukemia were 2.43 (95% CI 0.81 – 7.25) for top exposures of 2 – 3.9 ppm and 3.46 (95% CI 1.27 – 9.43) for top exposures ≥4 ppm, compared to the reference category (0.1 – 1.9 ppm). For average exposures, the RRs for myeloid leukemia were 1.15 (95% CI 0.41 – 3.23) for 0.5 – 0.9 ppm and 2.49 (95% CI 1.03 – 6.03) for ≥1 ppm, compared to the reference category (0.1 – 0.4 ppm). (Newer calculations have yielded somewhat lower risk elevations for myeloid leukemia, see Reference 36.) In another industrial cohort (the NIOSH cohort) the SMR for myeloid leukemia was 1.44 (95% CI 0.80 – 2.37), in comparison to the entire population. For workers with both ≥10 years of exposure and ≥20 years since initial exposure the SMR was 2.55 (95% CI 1.10 – 5.03) for myeloid leukemia (analysis of multiple causes of death). Average exposure during the early 1980s was 0.09 – 0.20 ppm, but it may previously have been much higher (58, 105). In a recently published case-control study of a cohort of dead people in the undertaking business (47) the OR for myeloid leukemia among persons who had at some time worked with embalming was 11.2 (95% CI 1.3 – 95.6; p 0.027). A significant trend (p 0.02) was seen for myeloid leukemia with increasing number of years with embalming work. For the categories >0 – 20, >20 – 34, and >34 years the ORs were 5.0 (95% CI 0.5 – 51.6), 12.9 (95% CI 1.4 – 117.1) and 13.6 (95% CI 1.6 – 119.7). A significant trend was also noted for myeloid leukemia and top exposures (p 0.036): the ORs were 15.2 (95% CI 1.6 – 141.6) for >0 – 7.0 ppm, 8.0 (95% CI 0.9 – 74.0) for >7.0 – 9.3 ppm, and 13.0 (95% CI 1.4 – 116.9) for >9.3 ppm, compared to the reference (0 ppm). Calculated exposures for persons who had done embalming (cases and controls) were 0.1 – 0.2 ppm for 8-hour averages and 1.5 – 1.8 ppm during embalmings. Calculated top exposures were 8.1 – 10.5 ppm. In a meta-analysis (150) focused on the groups with highest exposure in 15 studies, the relative risk for leukemia was calculated to be 1.54 (95% CI 1.18 – 2.00; p<0.001). The highest relative risk was noted for myeloid leukemia (RR 1.90, 95% CI 1.31 – 2.76; p 0.001), based on 6 studies: 2 of industrial cohorts (45, 105) and 4 of embalmers/anatomists.

Elevated mortality due to cancers in the upper part of the throat (nasopharyngeal cancers) was reported in the NCI cohort: SMR 2.10 (95% CI 1.05 – 4.21; exact 95% CI 0.91 – 4.14), based on 8 exposed cases, compared to the population as a whole (46). The relative risk for death due to nasopharyngeal cancer was 1.83 in

the group with highest top exposures (≥4 ppm), and all the exposed cases were in this category. The RRs were 1.67 for the highest average exposure (≥1 ppm) and 4.14 for the highest cumulative exposure (≥5.5 ppm-years). There were statistically significant trends for both top exposure ($p<0.001$) and cumulative exposure ($p<0.025$). Most of the exposed cases (6 cases) occurred at the same factory, and the NCI-based median value for average exposure intensity there was a bit over 1 ppm. Two other large cohort studies of industrial workers showed no elevation in mortality due to nasopharyngeal cancer (26, 105). Many epidemiological studies, however, report neither observed nor expected deaths due to nasopharyngeal cancer (e.g. studies of embalmers and anatomists/pathologists). The study by Hauptmann *et al.* (47, see above), using cause-of-death records for people who had worked in the undertaking business, reports 4 cases of nasopharyngeal cancer, but only 2 of them had worked with embalming (OR 0.1, 95% CI 0.01 – 1.2).

Experimental data indicate that both genotoxicity and cytotoxicity play major roles in development of nasal cancer with exposure to formaldehyde. DNA-protein crosslinks in nasal mucosa have been observed with short-term exposures of ≥0.3 ppm for rats and ≥0.7 ppm for monkeys. The concentration-response curve for formation of crosslinks is biphasic, with increased steepness around formaldehyde levels of 2 – 3 ppm (20, 21, 58). Cell proliferation has been shown to increase considerably at air levels around 6 ppm, and the increased cell proliferation seems to greatly enhance the genotoxic effect of formaldehyde (58). Squamous cell carcinoma has been observed in nasal epithelium of rats at formaldehyde levels of about 6 ppm, with large increases in incidence at levels ≥10 ppm (65, 66, 95). The NOEL for squamous cell carcinoma in nasal epithelial cells was 2 ppm (95). However, inflammation of nasal mucosa, basal cell hyperplasia and squamous cell metaplasia in nasal respiratory epithelium, as well as polyp-like adenomas in the nasal cavity, have been reported with long-term exposure to 2 ppm (66). The RD_{50} (50% reduction in respiratory rate, a measure of sensory irritation) is in the range 3 – 5 ppm for mice and 20 – 30 ppm for rats (31, 33). Increased airway reactivity has been reported in guinea pigs with 8 hours of exposure to 0.3 ppm (125).

In behavior tests with mice, declines of spatial learning and memory have been reported at 2.4 ppm. At this air level there was an increase of MDA and reduction of SOD and GSH in the brain (82). In mice given intraperitoneal injections of formaldehyde that affected performance in behavior tests measuring spatial learning and memory, the formaldehyde level in the brain was significantly elevated (131). Poor performance in behavior tests, which the authors regarded as an expression of neurotoxicity, was reported in rats after repeated inhalation exposure to 2.6 ppm (106). The reason for the observed effects on behavior is not clear, however. Effects on vision and olfaction due to irritation of some sort has been proposed as a possible cause (58).

Effects on testes and sperm have been reported in inhalation studies in which rats were exposed to 5 – 8 ppm (152, 155).

Observed effects on animals exposed to formaldehyde by inhalation are summarized in Table 3.

Conclusions

The critical effects of occupational exposure to formaldehyde are irritation of mucous membranes and genotoxicity. Slight eye irritation has been reported by volunteers exposed briefly to 0.2 – 0.3 ppm, and at air levels around 0.4 ppm (0.2 – 0.6 ppm) symptoms of nasal irritation are quite apparent (sneezing, itching, swollen mucous membranes, elevated numbers of white blood cells in nasal mucosa). Increased airway reactivity at 0.3 ppm has also been observed in experimental animals. With occupational exposures, genotoxic effects have been observed at average levels around 0.1 – 0.4 ppm with brief periods of higher exposure.

Formaldehyde is genotoxic and carcinogenic to humans. Elevated incidences of nasopharyngeal cancer have been observed in epidemiological studies, and formaldehyde causes tumors in the nasal cavities of rats. Elevated incidences of myeloid leukemia have also been observed in epidemiological studies.

DNA damage has been observed in nasal mucosa of rats at air levels ≥0.3 ppm. Experimental data indicate that irritative cytotoxicity and cell proliferation are prerequisites for the appearance of cancer in upper respiratory passages. Exposure that does not have these effects, i.e. 0.2 – 0.3 ppm, should therefore not cause cancer in respiratory passages. However, it has not been shown that the appearance of leukemia is dependent on these effects.

Formaldehyde is a contact allergen, and causes allergic contact eczema.

Table 2. Effects of formaldehyde on volunteers in exposure chamber studies (group averages, subjective assessments unless otherwise noted).

Air level (ppm)	Exposure time	Subjects	Response (%)	Effects	Ref.
0.07	30 min.	19 asthmatics with mite allergy	n/a	Significantly lower average induction dose of mite allergen needed for 20% reduction of FEV_1 on bronchial provocation (immediate reaction), significant reduction in maximum FEV_1 (15% vs. 11%) within 6 hours after bronchial provocation with mite allergen.	24
0.15	4 hrs.	21	–	No effects.	73
0.24	5 hrs.	16	19	Very slight discomfort (irritated conjunctiva, dryness in nose and mouth) (rank 2-9/100[a]).	1
0.3	4 hrs.	21	Not reported	Very slight eye irritation (max. rank 0.6/5[b]), very slight respiratory irritation (max. rank 0.5/5[b]).	73
0.3 + 4 x 0.6	4 hrs.	21	Not reported	Very slight eye irritation (max. rank 1.1/5[b]) (significant only compared to EA controls[d]).	73
0.35	6 min.	12	42 (not significant)	Very slight eye irritation (rank 0.7/3[c]).	13
0.4	5 hrs.	16	31	Very slight discomfort (irritated conjunctiva, dryness in nose and throat) (rank 2-5/100[a]).	1
0.4 (0.16-0.57)	2 hrs.	10 healthy, 10 with inflamed nasal mucosa and asthma	Not reported	Nasal symptoms (sneezing, itching, inflamed mucosa); increase of total leukocytes and eosinophilic leukocytes, elevated albumin/total protein ratio in nasal lavage.	70
0.4	2 hrs.	11 healthy, 9 skin-sensitized to HCHO	Not reported	Nasal symptoms (sneezing, itching, inflamed mucosa), increase of eosinophilic cells and protein (albumin, total protein) in nasal lavage.	104
0.5	3 hrs.	10	10	Very slight nose/throat irritation (rank 0.1/3[c]).	7, 71
0.5	4 hrs.	21	Not reported	Very slight eye irritation (max. rank 0.6/5[b]).	73
0.5 + 4 x 1	4 hrs.	21	Not reported	Significant increase in average blinking frequency ($p<0.05$), increase of moderately reddened conjunctiva (80% vs. 40%). Very slight eye irritation (max. rank 1.5/5[b]), slight nasal irritation (max. rank 2/5[b]), very slight respiratory irritation (max. rank 0.7/5[b]).	73
0.5 + 4 x 1 + EA[d]	4 hrs.	21	Not reported	Significant increase in average blinking frequency ($p<0.05$). Very slight eye irritation (max. rank 1.6/5[b]), slight nasal irritation (max. rank 2/5[b]), very slight respiratory irritation (max. rank 0.8/5[b]).	73

Table 2. Continued.

Air level (ppm)	Exposure time	Subjects	Response (%)	Effects	Ref.
0.56	6 min.	26	54 (not significant)	Very slight eye irritation (rank 0.8/3[c]).	13
0.7	6 min.	7	57 (not significant)	Very slight eye irritation (rank 0.9/3[c]).	13
0.8	5 hrs.	16	94	Very slight discomfort (irritated conjunctiva, dryness in nose and throat) (rank 2-10/100[a]).	1
0.9	6 min.	5	60 (not significant)	Very slight eye irritation (rank 0.8/3[c]).	13
1	1.5 min.	48	Not reported	Very slight nasal irritation (rank 1.5/4[e]).	138
1	6 min.	27	74	Slight eye irritation (rank 1.6/3[c]).	13
1	3 hrs.	19	21 mild 5 moderate	Very slight eye irritation (rank 0.3/3[c]).	7, 71
			5 mild	Very slight nose/throat irritation (rank 0.05/3[c]).	
1.2[f]	35 min.[f]	33	Not reported	Very slight eye irritation (rank 1.5/4[e]), very slight nasal irritation (rank 1.4/4[e]).	138
1.6	5 hrs.	16	94	Very slight discomfort (irritated conjunctiva, dryness in nose and throat) (rank 2-18/100[a]).	1
1.7[f]	35 min.[f]	33	Not reported	Significantly higher average blinking frequency. Very slight eye irritation (rank 1.7/4[e]), very slight nasal irritation (rank 1.5/4[e]).	138
2	1.5 min.	48	Not reported	Very slight eye irritation (rank 1.4/4[e]), slight nasal irritation (rank 2.1/4[e]), very slight throat irritation (rank 1.4/4[e]).	138
2	3 hrs.	19	32 mild 21 moderate	Very slight eye irritation (rank 0.7/3[c]).	7, 71
			37 mild	Very slight nose/throat irritation (rank 0.4/3[c]).	

[a] Group average at different points in time. Highest individual rankings 30/100 at 0.24 ppm, 20/100 at 0.4 ppm, 40/100 at 0.8 ppm, 50/100 at 1.6 ppm. 0 = no discomfort, 1-33 = slight discomfort, 34-66 = discomfort, 67-99 = strong discomfort 100 = intolerable.

[b] Group average at time with maximum rankings: 0 = no discomfort, 1 = slight discomfort, 2 = somewhat, 3 = quite, 4 = strong, 5 = very strong.

[c] 0 = none, 1 = slight, 2 = moderate, 3 = strong.

[d] 13 ppm ethyl acetate.

[e] 1 = none at all, 2 = slight, 3 = moderate, 4 = strong.

[f] 0 to 3.2 ppm, gradually rising air concentration.

Table 3. Effects noted in laboratory animals exposed to formaldehyde by inhalation.

Exposure		Species	Effects	Ref.
ppm	time			
0.2	22 hours/day, 26 weeks	Rat Monkey Hamster	No noteworthy effects.	109
0.3	10 minutes	Mouse	RD_{10}	31
0.3	6 hours	Rat	Significant increase of DNA-protein crosslinks in nasal mucosa.	20, 58
0.3	8 hours	Guinea pig	Increased airway reactivity.	125
0.4	6 hours/day, 21 days	Rat	Increased airway reactivity, increased numbers of eosinophilic cells in bronchoalveolar lavage from animals sensitized with OVA allergen.	108
0.6	6 hours/day, 5 days/week, up to 3 weeks	Rat	No histopathological changes in nasal mucosa, no significant changes seen in genome analysis.	4
0.7	6 hours	Monkey	Increase of DNA-protein crosslinks in nasal mucosa.	21, 58
0.8	24 hours/day, 5 days/week, 5 weeks	Mouse	Elevated OVA-specific IgE antibody formation ($p<0.05$) in animals sensitized with OVA allergen.	41
1	22 hours/day, 26 weeks	Rat Monkey Hamster	Squamous cell metaplasia/hyperplasia in nasal turbinates of 1/6 monkeys. No noteworthy effects observed in rats and hamsters.	109
1	6 hours/day, 5 days/week, 28 months, or 3 months with 25 months of observation	Rat (undamaged nasal mucosa)	Somewhat reduced weight gain ($p<0.05$).*	145
1.2	4 hours/day, 4 months, prior to mating	Rat (females)	Significant increase in numbers of degenerated embryos.	58**
1.8	6 hours/day, 5 days/week, up to 3 weeks	Rat	Somewhat higher incidence of epithelial hyperplasia in noses, minimal changes in gene expression seen in genome analysis.	4
2	6 hours/day, 5 days/week, 1, 2, 4 or 9 days	Rat	Minimal clinical indications of eye and nose irritation, no epithelial damage in nasal mucosa (histopathological examination), inhibited cilial activity in portions of nasal mucosa in some animals with 9 days of exposure.	96
2	8 hours/day, 5 days/week, 13 weeks	Rat	NOAEL in the study.	142
2	6 hours/day, 5 days/week, 2 years	Rat	NOAEL in the study for squamous cell carcinoma, polyp-like adenoma, cell proliferation and non-neoplastic changes in nasal cavity (inflammatory changes, squamous cell metaplasia, hypertrophy/ hyper-plasia).	95

Table 3. Continued.

Exposure		Species	Effects	Ref.
ppm	time			
2	6 hours/day, 5 days/week, 2 years	Rat Mouse	Rat: LOAEL in the study. Polyp-like adenomas in nasal cavities with significant ($p<0.05$) trend for males (3.4% vs. 0.4% in controls); non-neoplastic changes furthest out in the nose (inflammation in nasal mucosa, epithelial dysplasia, squamous cell metaplasia in respiratory epithelium), squamous cell metaplasia in nearly 100%. Mouse: NOAEL in the study (a few animals with inflammation in nasal mucosa).	7, 66
2	6 hours/day, 5 days/week, 28 months	Rat	Elevated incidence of squamous cell metaplasia/ epithelial cell hyperplasia in nasal cavity.	65
2	6 hours/day, days 6-15 of gestation	Rat	NOEL in the study.	32, 58, 91
2.4	6 hours/day, 7 days	Mouse	Negative effects on spatial learning and memory in behavior tests, increase of MDA and reduction of SOD and GSH in brain, significant increase in gene expression of NMDA transmitter-receptors (NR1 and NR2B mRNA) in brain.	82
2.5	6 hours/day, 21 days	Rat	Increased airway reactivity; IFN-γ increased and IL-4 reduced in lungs.	108
2.6	10 min./day, 7 days/week, 90 days	Rat	Worse performance in behavior tests: significantly longer to find food in a maze from week 7, significantly higher number of mistakes week 12 (no dose-response).	106
3	6 hours/day, 5 days/week, 13 weeks	Rat	Increased cell proliferation and squamous cell metaplasia (with or without keratinization) in respiratory epithelium in area furthest out in nose.	153
3	22 hours/day, 26 weeks	Rat Monkey Hamster	Rat: increased occurrence of inflammation in nasal mucosa, squamous cell metaplasia/hyperplasia and basal cell hyperplasia in nasal turbinates; reduced weight gain, reduced absolute and relative liver weights. Monkey: increased incidence of hoarseness, nasal discharge; squamous cell metaplasia/hyperplasia in nasal turbinates of 6/6 animals. Hamster: no significant responses.	109
3.1 - 5.3	5 to 10 minutes	Mouse	RD_{50}	31
3.6	8-hour exposures with 4-hour exposure-free intervals, 72 hours	Rat	Increased cell proliferation in nasal respiratory epithelium; degeneration, necrosis, hyperplasia/ squamous cell metaplasia in respiratory epithelium, inflammation in nasal mucosa.	23

Table 3. Continued.

Exposure		Species	Effects	Ref.
ppm	time			
4	4 hours/day, 5 days/week, 13 weeks (8 x 30-minute exposures/day separated by 30 exposure-free minutes)	Rat	Increased degree and incidence of histopathological changes in nasal respiratory epithelium (including focal squamous cell metaplasia).	142
4.9	8 hours/day, 5 days/week, 4 or 13 weeks	Rat	Significantly higher levels of zinc and copper in cerebral cortex, reduced weight gain.	154
5	6 hours/day, 5 days/week, up to 3 weeks	Rat	Significantly increased cell proliferation in nasal epithelium day 5 (reduced in degree and extent day 15); inflammatory cell infiltration, hyperplasia and squamous cell metaplasia in respiratory and transitional epithelium; changes in expression of several different genes in genome analysis.	4
5	8 hours/day, 5 days/week, 13 weeks	Rat	Reduced serum testosterone levels, reduced epididymal diameter, increased synthesis of a stress marker (heat shock protein 70) in sperm, irritation effects in nasal mucosa, reduced feed and water intake.	155
5	6 hours/day, days 6-15 of gestation	Rat	No indications of maternal toxicity; significantly reduced ossification (pelvic girdle) (associated to larger litters and lower fetal weights); no increase in incidence of litters/fetuses with developmental defects/anomalies; no effects on sex ratio, fetal weight, number of corpora lutea, implantation locations, resorptions or living fetuses.	32, 58, 91
5.1	6 hours/day, 5 days/week, 2 weeks	Rat	DNA damage (strand breaks) in lung cells, peripheral lymphocytes and liver cells; cytokines IL-4 upregulated and IFN-γ downregulated (plasma).	61, 120
5.6	6 hours/day, 5 days/week, 2 years	Rat Mouse	Rat: squamous cell carcinoma in nasal cavities of 0.9% (not significant); polyp-like adenomas in nasal cavities with significant ($p<0.05$) trend for males (5% vs. 0.4% in controls); non-neoplastic changes (inflammation in nasal mucosa, epithelial dysplasia and squamous cell metaplasia in respiratory epithelium); reduced weight gain. Mouse: LOAEL in the study. A few animals with dysplasia, metaplasia and inflammatory changes in respiratory epithelium, focal atrophy of olfactory epithelium in a few animals.	7, 66

Table 3. Continued.

Exposure		Species	Effects	Ref.
ppm	time			
6	6 hours/day, 5 days/week, 2 years	Rat	LOAEL in the study. Squamous cell carcinomas in nasal cavities of 1.1%, no animals with polyp-like adenomas; slight increase of persistent cell proliferation in nasal epithelial cells (taking into consideration the size of the cell population), minimal focal squamous cell metaplasia in nasal cavities.	95
8	12 hours/day, 2 weeks	Rat	Reduced testes weight, histopathological and biochemical changes (lower GSH, GSH peroxidase and superoxide dismutase, higher malondialdehyde) in testes, increased proportion of abnormal and reduced proportion of motile sperm, lower sperm counts.	152
9.4	2 hours	Guinea pig	Increased airway reactivity.	125
9.8 or 9.2	6 hours/day, 5 days/week, 28 months, or 3 months with 25 months of observation	Rat (undamaged nasal mucosa)	With 3 and 28 months of exposure: inflammation in nasal mucosa and squamous cell metaplasia in nasal respiratory epithelium (at 28 months also basal cell hyperplasia in respiratory epithelium and thinning of olfactory epithelium); reduced weight gain; not-significant increase of nasal tumors.	145
9.9	6 hours/day, 5 days/week, 2 years	Rat	Squamous cell carcinoma in nasal cavities of 22%, polyp-like adenomas in noses of 5.6%, persistent and significant increase of cell proliferation in nasal epithelium, hypertrophy/hyperplasia, squamous cell metaplasia and inflammatory changes in respiratory epithelium.	95
10.2 or 10.6	6 hours/day, 1 or 5 days	Rat	DNA adducts in nasal mucosa (due to exogenous formaldehyde exposure) but not in other examined tissues.	81
10	6 hours/day, days 6-20 of gestation	Rat	NOEL in the study.	32, 58, 110
10	8 hours/day, 5 days/week, 4 weeks	Rat	Significant reduction of GSH concentration in liver.	126
10	8 hours/day, 5 days/week, 4 or 13 weeks	Rat	Significant increase of SOD activity in heart tissue (both exposure times), reduced catalase activity in heart tissue (significant only after 4 weeks).	42
10.1	6 hours/day, 5 days/week, 2 weeks	Rat	DNA damage (strand breaks) in peripheral lymphocytes and liver cells; significant increase in lipid peroxidation (measured as MDA) and protein oxidation in plasma and liver, cytokines IL-4 upregulated and IFN-γ downregulated (plasma).	61

Table 3. Continued.

Exposure		Species	Effects	Ref.
ppm	time			
14.3	6 hours/day, 5 days/week, 2 years	Rat Mouse	Rat: squamous cell carcinomas in nasal cavities of 44%; polyp-like adenomas in nasal cavities with significant ($p<0.05$) trend for males (3.4% vs. 0.4% in controls); non-neoplastic changes (inflammation in nasal mucosa, dysplasia and squamous cell metaplasia in respiratory epithelium), weight loss, breathlessness. Mouse: squamous cell carcinoma in 1.7%, (males, not significant); inflammatory changes, dysplasia and squamous cell metaplasia in respiratory epithelium, focal atrophy of olfactory epithelium.	66
15	6 hours/day, 5 days	Rat	No significant increase of chromosome aberrations or SCE in lymphocytes.	68
15	6 hours/day, 5 days/week, 4 weeks	Rat	No significant effects in genotoxicity tests of blood (micronuclei in peripheral blood, SCE in lymphocytes, DNA strand breaks and DPC in leukocytes) or alveolar cells (micronuclei, DNA strand breaks, DPC).	97, 118
15	6 hours/day, 5 days/week, 1 or 8 weeks	Rat	Significant increase of chromosome aberrations in alveolar macrophages (after 1 and 8 weeks); no significant increase of chromosome aberrations in bone marrow cells.	30
15	6 hours/day, 5 days/week, 2 years	Rat	Squamous cell carcinoma in nasal cavities of 47%, polyp-like adenomas in noses of 9.5%, sustained, significant increase of cell proliferation in nasal epithelium, hypertrophy/hyperplasia, squamous cell metaplasia and inflammatory changes in respiratory epithelium.	95
15	6 hours/day, 5 days/week, 28 months	Rat	Squamous cell carcinoma in nasal cavities of 41%, squamous cell papillomas in nasal cavities of 9%, increased incidence of squamous cell metaplasia/ epithelial cell hyperplasia and epithelial cell hyperkeratosis in nasal cavities; reduced feed consumption, weight loss, reduced liver weight, no hematological effects.	65
20	6 hours/day, days 6 to 20 of gestation	Rat	Reduced fetal weight (males only), no significant differences for other examined parameters (gravidity incidence, number of implantations, prenatal death, developmental defects, sex ratio).	32, 58, 110

MDA = malondialdehyde, SOD = superoxide dismutase, OVA = ovalbumin

* In animals with intentionally damaged (by electrocoagulation) nasal mucosa, however, body weights at these exposures were somewhat higher than controls.

** Kitaeva *et al.* 1990, reviewed in Reference 58.

Potential conflicts of interest

Gunnar Johanson (member of the Criteria Group) has reported that he collaborated on the assessment of formaldehyde and recommendations regarding health-based occupational exposure limits made by the Scientific Committee on Occupational Exposure Limits (SCOEL) at the request of the EU.

Per Gustavsson (member of the Criteria Group) has reported that he collaborated on the IARC assessment of formaldehyde in 2009.

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Consensus Report for Organic Acid Anhydrides

September 29, 2010

This Consensus Report constitutes a basis for assessing the risks of occupational exposure to the organic acid anhydrides phthalic anhydride (PA), trimellitic anhydride (TMA), maleic anhydride (MA), hexahydrophthalic anhydride (HHPA), methylhexahydrophthalic anhydride (MHHPA), methyltetrahydrophthalic anhydride (MTHPA), tetrahydrophthalic anhydride (THPA) and tetrachlorophthalic anhydride (TCPA). The present document is based on a criteria document produced jointly by the Nordic Expert Group and the Dutch Expert Committee (8, 27), and contains additional information from literature searches made through January of 2010. This document is an update of the consensus report for organic acid anhydrides published in 2009 (39). The reason for this new assessment after such a brief interval is that some important studies (18, 50) have now been included. The Swedish Criteria Group also published a previous Consensus Report on organic acid anhydrides in 1991 (36).

Chemical and physical data. Occurrence

Anhydride	*CAS No.*	*Abbreviation*	*Formula*	*Mol weight*
Phthalic anhydride	85-44-9	PA	C_8H4O_3	148.12
Trimellitic anhydride	552-30-7	TMA	$C_9H_4O_5$	192.13
Maleic anhydride	108-31-6	MA	$C_4H_2O_3$	98.06
Hexahydrophthalic anhydride	85-42-7	HHPA	$C_8H_{10}O_3$	154.17
Methylhexahydrophthalic anhydride	25550-51-0	MHHPA	$C_9H_{12}O_3$	168.19
Methyltetrahydrophthalic anhydride	26590-20-5	MTHPA	$C_9H_3O_3$	166.19
Tetrahydrophthalic	117-08-8	TCPA	$C_8Cl_4O_3$	285.88

Some physical and chemical data are presented in Table 1, and structures are shown in Figure 1. For PA there is a reported octanol/water distribution coefficient, log P_{ow} = - 0.62 (i.e. more soluble in water than in octanol), and a reported odor threshold of 320 µg/m^3. For MA the reported odor threshold is 1230 µg/m^3. Further data and synonyms are presented in the Criteria Document (27).

Table 1. Some chemical and physical data on organic acid anhydrides. Further data are presented in the Criteria Document (27).

Anhydride	State at room temperature	Melting point (°C)	Boiling point (°C)	Vapor pressure (Pa)	Saturation concentration (ppm
PA	Crystalline	130.8	284 (subl.)	<6.6 at 20°C	<65
TMA	Crystalline	161 – 163.5	240 – 245	<10 at 25°C	<99
MA	Crystalline	53	202 (subl.)	25 at 20°C	250
HHPA	Crystalline	35 – 36	158 (2.3 kPa)		
MHHPA	Liquid	- 29	120 (130 Pa)		
MTHPA	Liquid		>200	0.1 at 20°C	1
THPA	Crystalline	101.9	195 (6.7 kPa)	1.3 at 20°C	13
TCPA	Crystalline	254 – 255	371 (subl.)		

(subl.) = sublimates

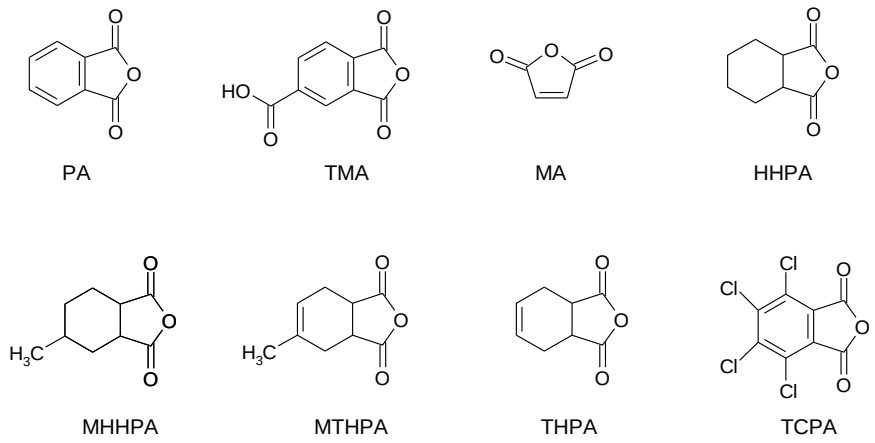

Figure 1. Structural formulas for the above organic acid anhydrides.

Organic acid anhydrides are used primarily in the production of polyester and alkyd plastics (PA, TMA, MA, THPA, TCPA) and as hardeners in production of epoxy plastics (PA, HHPA, MHHPA, MTHPA). Organic acid anhydrides do not occur naturally. Occupational exposure occurs around production and handling of the compounds. In Sweden, the highest levels in workplace air have been measured around reactors being charged with the substances (PA, MA) in powder form, and around operations that involve heating of free organic acid anhydrides (HHPA, MHHPA, MTHPA). The organic acid anhydrides have low vapor pressures (Table 1), and exposure is therefore primarily to airborne particles. Particles are formed by condensation/sublimation at room temperature after the substances have been heated.

A representative selection of air levels recorded with personal monitors in Swedish companies (unless otherwise stated) is presented below:

PA. Levels up to 17,000 μg/m^3 have been measured around reactors being charged with PA powder for production of alkyd and/or unsaturated polyester resins. The time-weighted average (TWA) for a workshift was estimated to be about 400 μg/m^3 (40). Considerably lower averages (38 μg/m^3) were measured in a later English study (58). Low levels of PA are also released during processing of PVC plastics that contain phthalates as softeners (57).

TMA. In Sweden, levels in the range 6 – 180 μg/m^3 have been measured around powder painting (7). In the English study mentioned above, levels up to 20,000 μg/m^3 were measured during charging of reactors; levels around other operations were usually below 40 μg/m^3 (58).

MA. 600 μg/m^3 has been recorded around reactors being charged with MA (41). A Finnish study (46) reports extremely high levels of MA in air during reactor charging in a factory that synthesized polyester plastic. The highest MA level measured (44-minute sample) was 23,500 μg/m^3. Five years later, after the factory was modernized, the measured level was 1200 μg/m^3. In another factory, where thermoplastic was polymerized, air levels of MA were measured on several occasions over a ten-year period. On the first occasion the average MA level was 219 μg/m^3 (range 5.8 – 470 μg/m^3, average sampling time 6.9 hours, n = 7). Levels then gradually dropped as process improvements (not described) were introduced, and ten years later measurements showed 5.8 μg/m^3 (range <0.4 – 24 μg/m^3, average sampling time 6.6 hours, n = 6).

THPA. No exposure data for this compound were found in the literature.

HHPA and **MHHPA**. These compounds are usually handled simultaneously in mixing. In two companies that insulate electronic components, HHPA levels up to 470 μg/m^3 were measured around casting. Daily averages recorded by personal monitors were 23 – 140 μg/m^3 for HHPA and 9 – 48 (with a peak value of 403) μg/m^3 for MHHPA (60). In a Japanese company using mostly HHPA, but also some MHHPA, average levels of HHPA were below 40 μg/m^3 (68).

MHHPA was monitored in two electronics companies in Finland (47). Average exposures ranged from 68 to 118 μg/m^3 for workers in condenser production, and levels up to 1900 μg/m^3 were recorded around work at the ovens. The highest exposure measured at the company was 2200 μg/m^3 (8-hour average). Levels were also quite high in adjacent offices (17 – 43 μg/m^3).

MTHPA. In a company producing barrels for grenade rifles, MTHPA levels were up to 380 μg/m^3 with an average value of 100 μg/m^3 for the most highly exposed group (59). Levels recorded in two Japanese companies were 5 and 26 – 64 μg/m^3 (66).

Exposures to MTHPA and exposures to HHPA and MHHPA combined were monitored in a prospective study with 163 participants, made during 1988 – 1997. Exposure levels were derived from monitoring data and job descriptions. Average exposure was calculated to be 15.4 μg/m^3 (TWA), with a range from below the detection limit (1 μg/m^3) to 189 μg/m^3 (62).

TCPA: Exposure levels of 140 – 590 μg/m^3 are reported in a study made at a Canadian company. Levels dropped to below 110 μg/m^3 after intervention measures were taken (35).

Uptake, biotransformation, excretion

When volunteers were exposed to HHPA in gas form (80 μg/m^3) for 8 hours, 1 – 4% was recovered in exhaled air (25). In another study, more than 85% of absorbed HHPA was recovered as hexahydrophthalic acid (HHP acid) in urine (23). In a study with 3 volunteers, 1400 μg HHPA dissolved in vaseline (2% HHPA) was placed on a total area of 2 cm^2 skin under occlusion for 48 hours: estimated uptakes were 1.4 – 4.5%, 0.2 – 1.3%, and 0 – 0.4% (24). Uptakes were determined by collecting urine at 4-hour intervals for a total of 72 hours and analysis for HHP acid. The authors concluded that uptake of organic acid anhydrides by healthy skin is low.

Lindh *et al.* (34) used autoradiography to map the distribution of radioactively labeled HHPA in guinea pigs and rats after 3 to 8 hours of inhalation exposure. Medium-high to high levels of radioactivity were seen in nasal and tracheal mucosa, whereas levels in lung tissue were minimal. Tissue-bound radioactivity was also seen in the digestive tract and conjunctiva, and low levels in the renal cortex (rats only). The animals still contained some radioactivity at least 7 days after the exposure. With the exception of the lungs, the radioactivity was only partially extractable with organic solvents and water, which indicates that it was covalently bound. The radioactivity in dialyzed plasma was found mostly in the albumin fraction (gel filtration).

The anhydride group reacts rather quickly with amino acids and is conjugated with plasma proteins (27). Adducts in plasma proteins, including human serum albumin (HSA), have been measured in serum from workers exposed to HHPA and MHHPA. The half time for the adducts was about 20 days (48). Conjugation with hemoglobin was observed when human erythrocytes were exposed to HHPA and MHHPA (33). Organic acid anhydrides react with water to form the corresponding carboxylic acids, and are excreted in this form in urine. In persons occupationally exposed to 1630 μg/m^3 PA there was a rise in pre-shift urine concentration of phthalic acid during the workweek. The half time for PA in urine is 14 hours. The half time for HHPA is 2 – 3 hours in urine and 1.7 – 1.8 hours in plasma. For workers exposed to industrial MTHPA, excretion time in urine ranges from 3.3 to 6 hours for the four isomers (27).

Biological exposure monitoring

Levels of HHP acid in plasma and urine of exposed volunteers have shown good correlations with air concentrations. The half time for MHHPA in urine of exposed workers ranged from 4 to 10 hours. There was a strong linear and proportional correlation between air levels and the corresponding concentrations in urine. Exposure to 20 μg/m^3 (8-hour average) corresponds to a level of 140

nmol MHHP acid/mmol creatinine in urine, and to about 40 nmol/l in hydrolyzed plasma (32). Similar studies of MTHPA-exposed persons in Japan showed that excretion of MTHP acid in urine increased during exposure and then declined, with a half time of about 3 hours. An exposure of 50 μg/m^3 (8-hour average) corresponded to an excretion of about 900 nmol/mmol creatinine in urine (70). Total plasma protein adducts of HHPA and MHHPA showed a strong correlation to exposure as measured in the form of urinelevels of HHP acid and MHHP acid. The half times for the protein adducts in plasma were 22 days for HHPA and 24 days for MHHPA. MHHPA yields about three times the amout of protein adducts in plasma as HHPA at the same exposure level (49). Exposure to HHPA measured as HHP acid in urine correlated well with HHPA-hemoglobin adducts in blood (27).

Available data indicate that it should be possible, for some of these substances (especially MHHPA and HHPA), to express a correlation between exposure and effect in the form of acid levels in urine or protein adducts in plasma. Such a procedure would be attractive since it provides an integrated measure of dose over a longer time and includes exposure via inhalation as well as skin uptake, and would thus show the effects of protective equipment.

Toxic effects

Human data

Organic acid anhydrides are irritating to skin and to mucous membranes of eyes and respiratory organs, and cause symptoms such as itching, watery eyes, sneezing, runny noses, coughing and breathlessness (27).

Rhinoconjunctivitis and/or asthma have been observed in workers exposed to every one of the organic acid anhydrides reviewed here except THPA. Both immediate and late reactions, as well as a combination of the two, have been demonstrated in provocation tests with PA, MA, HHPA, MTHPA and TCPA. The Criteria Document contains reviews of several crosssectional studies presenting correlations between asthma and industrial use of organic acid anhydrides (27).

Work-related respiratory problems, including asthma, may occur in exposed workers with IgE antibodies specific for organic acid anhydrides (sensitized) as well as in workers without such antibodies. It has therefore been assumed that there are different mechanisms behind the respiratory symptoms induced by organic acid anhydrides: one mechanism with an IgE-mediated allergic reaction (3, 26, 27) and another IgE-independent specific hypersensitivity reaction of unclear nature. The latter may be due to a cell-mediated allergy (20, 22). Non-immunological mechanisms resulting from irritative/toxic effects can also result in respiratory symptoms (1, 37, 42). Since correlations between IgE and respiratory symptoms have been examined almost exclusively in cross-sectional studies, however, any such correlations may have been masked or underestimated.

There are considerable data associating asthma or rhinitis with the presence of IgE antibodies specific for all of the organic acid anhydrides discussed here except

THPA. Sensitization has been identified by skin prick test and by IgE antibodies in serum against HSA conjugates of organic acid anhydrides. The biological half time for the IgE antibodies after exposure is stopped is about 1 year (27). In a prospective study, Nielsen *et al.* (45) found a significant correlation between symptoms and specific IgE antibodies, although not all the subjects with the IgE antibodies had symptoms. Since the study was halted by positive tests for antibodies specific for organic acid anhydrides, there is no way of knowing how many would have developed symptoms if exposure had been continued.

The question of a relationship between atopy or smoking and sensitization and symptom development with exposure to organic acid anhydrides has been addressed in numerous studies (3, 5, 6, 17, 35, 42, 44, 45, 55, 59, 60, 62, 63, 65, 69). Some studies show that smoking, and especially atopy, increase the risk of sensitization and symptom development, but the results are not consistent.

Although allergic contact eczema (Type IV allergy) has seldom been described, IgE-mediated contact urticaria is not uncommon. PA, MA, MHHPA, MTHPA and HHPA have been reported to cause contact urticaria in exposed workers with specific IgE antibodies and positive prick tests (27). Yokota *et al.* (67) report a case of urticaria caused by MHHPA, but not HHPA, after only airborne exposure to both anhydrides. In a review of 87 patients who had positive reactions to prick tests with acid anhydrides given by the Finnish Institute of Occupational Health during the years 1990 – 2006, it was noted that 21 had been diagnosed with contact urticaria caused by acid anhydrides. Three of the subjects had urticaria alone, whereas the others also had allergic rhinitis (16 persons) and/or asthma (5 persons). For eight of them the contact urticaria was triggered by airborne acid anhydride. Of the 21 diagnosed with contact urticaria, 11 were patch-tested for contact allergy to acid anhydrides. None of them showed a positive reaction (21). The authors concluded that symptoms of contact urticaria brought on by exposure to acid anhydrides are probably more common than previously believed.

PA and **MA.** Workers are often exposed to these two compounds simultaneously. In a study of 60 workers with top exposures up to 17,400 µg/m^3, Nielsen *et al.* (40) found that, of the 35 who had highest exposures (average about 400 µg/m^3), 5 had occupational asthma and 24 had occupational rhinitis (40%) and/or conjunctivitis (46%). Exposure time was on average 13 years. Of the 25 with lower exposures (below 100 µg/m^3), 5 had occupational rhinitis or conjunctivitis and none had asthma. Average exposure time for this group was 12 years. Air concentrations of PA were measured with personal monitors: a total of 29 samples with a total sampling time of 20 hours. One subject in the high-exposure group and three in the low-exposure group had PA-specific IgE antibodies. The results indicate a primarily non-IgE-mediated effect of PA. These persons were also exposed to other anhydrides, including MA, but to a much lesser extent.

In an English retrospective study of workers in three factories, who handled PA and MA and to some extent also TMA, there were 4 cases of sensitization among a total of 285 current and former employees (3). Exposures were measured with personal monitors, for entire shifts and also during some operations where high

levels might be expected. The measurements were made over a period of 2 to 4 weeks. There were 84 samples for PA, 39 for MA and 84 for TMA. Average exposures to PA in the three factories were 8.9, 61.9 and 11.9 μg/m^3. Levels of MA in 2 of the factories were 2.8 and 1.8 μg/m^3. TMA levels were 0.9, 0.7, and 0.5 μg/m^3.

There are only a few reported cases of MA-mediated asthma without simultaneous exposure to PA. In a study in which a provocation test was given to two MA-exposed workers, both early and late reactions to MA were obtained (16). In another study a worker who had developed asthma after one month of exposure to MA and PA had a positive reaction to provocation with MA but no reaction to provocation with PA (30).

TMA. Both immediate and late airway reactions have been reported. Symptoms of the late asthmatic reaction were coughing, wheezing and breathlessness 4 – 8 hours after the exposure. "Pulmonary disease-anemia syndrome," a specific lung disease which can develop into severe illness with hemorrhage, has also been described. Of 9 workers exposed to high levels of TMA (1700 and 3600 μg/m^3 are two time-weighted 8-hour averages) from powder paint, four had irritation-mediated effects and three had symptoms indicating an asthmatic reaction (31). During 1976 – 1987, examinations were given to 196 workers in a company that made TMA. IgE-mediated asthma or rhinitis was identified in 21 of them and late asthmatic reactions in 10. One had the pulmonary disease-anemia syndrome. Only 46 persons were entirely without symptoms (72). In a cross-sectional study made in the same factory in 1988 – 1989 a further 310 workers were examined, and 2 of 8 in the group with highest exposure to TMA (0.54 – 6500 μg/m^3; geometric mean 170 μg/m^3; based on records from 29 personal monitors) had TMA-specific IgE antibodies (73). In an examination of 25 workers with TMA-induced asthma, Grammer *et al.* (19) found that 88% had rhinitis, and 68% reported symptoms of conjunctivitis. The rhinitis had preceded the asthma in 77% of patients who had both rhinitis and asthma, and of those with both conjunctivitis and asthma 82% had developed the conjunctivitis first. Symptoms of rhinitis and conjunctivitis thus often precede the development of asthma.

In a factory where TMA was the only organic acid anhydride used, 107 workers were given prick tests to determine the presence of TMA-specific IgE antibodies. Personal monitors were used to measure whole-shift exposures, and additional readings were taken around operations where high exposures could be expected. The measurements were made over a period of 2 to 4 weeks, and 49 air samples were analyzed. The subjects were divided into three exposure groups: <10, 10 – 40, and >40 μg/m^3. Positive responses to the prick test were obtained in 1/63, 5/36 and 2/8 respectively. In addition, 12 subjects with work-related respiratory symptoms were each matched to 4 controls in a case-control study. Positive skin prick tests and work-related respiratory symptoms were significantly more common among the workers exposed to more than 10 μg/m^3 TMA than among those with lower exposures (3).

There is a study of 286 TMA-exposed workers in a factory that produced TMA (18). Every year for three successive years, the workers were checked for sensitization and the appearance of TMA-induced immunological disease (not further described). An occupational hygienist divided the workers into 5 exposure categories on the basis of job description, and air levels for a few persons in each category were obtained from personal monitors (no further information provided). Average air levels of TMA were 130 (range 2.9 –1700) μg/m^3 for group 1 (n = 28), 36 (2.3 – 1900) μg/m^3 for group 2 (n = 57), 2 (0.1 – 120) μg/m^3 for group 3 (n = 79), 0.51 (0.23 – 2.4) μg/m^3 for group 4 (n= 98), and <0.53 (<0.45– <0.6) μg/m^3 for group 5 (n = 24); see Table 2. In the three highest exposure groups there were respectively 7 (25%), 6 (11%) and 5 (6%) persons with TMA-specific IgE antibodies, and 9% of the workers in these three groups developed TMA-induced illness. No one in the two lowest exposure groups had specific IgE antibodies or became ill. The authors concluded that persons exposed to less than 2 μg TMA/m^3 are at low risk of developing a TMA-induced illness (18).

HHPA and **MHHPA**. In two Swedish companies where epoxy plastics containing HHPA and MHHPA were used to insulate electronic components, the highest levels of HHPA (130 and 470 μg/m^3) were measured around casting. MHHPA levels did not exceed 400 μg/m^3. The highest exposure average was 140 μg/m^3. Over a period of 2 years a total of 167 air samples were taken in the casting department, using both personal and stationary monitors. HHPA-specific antibodies were identified in 23 (24%) of the exposed workers (60).

In a factory that manufactured condensers, air levels of HHPA and MHHPA were measured with personal (122 samples, total sampling time 427 hours) and stationary (97 samples) monitors on 10 occasions during a one-year period. Work-related symptoms were significantly more frequent in exposed workers (n = 154) than in a reference group from a mechanical industry: eye symptoms 23% vs. 14%, nasal symptoms 28% vs. 16%, nosebleeds 8% vs. 0%, and symptoms involving the lower respiratory tract 10% vs. 4%. IgE antibodies specific for one or both anhydrides were identified in 34 (22%) of the subjects. The symptoms were correlated to exposure and to the antibodies. The workers were divided into three exposure categories based on current exposure (HHPA + MHHPA): <10, 10 – 50, and >50 μg/m^3. There were significant correlations between exposure and symptoms involving eyes, nose and lower respiratory tract. The proportion of workers with specific IgE antibodies was fairly high even in the low-exposure group (13% HHPA, 15% MHHPA), although the prevalence of symptoms was not significantly elevated (44). Asthma and rhinitis/conjunctivitis in workers exposed to HHPA had been reported in an earlier American study (38).

In a cross-sectional study by Rosqvist *et al*. (50), made in the same factory and with the same study population as the study by Nielsen *et al*. (44), protein adducts (TPPA, total plasma protein adducts) of HHPA and MHHPA in plasma were measured as biological exposure indicators. In some departments at the factory only HHPA was used, in some only MHHPA was used, and in the remaining departments both acid anhydrides were used in various proportions. Air levels

of HHPA had been reduced by half in the five years prior to the beginning of the study, and there had been a fivefold increase in air levels of MHHPA over the previous three years. The subjects (n = 139) had been employed for 4 years (median; range 0 – 29 years) and at the start of the study had been exposed for at least 4 months. IgE antibodies specific for the acid anhydrides were identified in 27 (19%) of them (the analysis did not differentiate between antibodies specific for HHPA and MHHPA), and work-related symptoms involving the nose (27%), eyes (21%) and lower respiratory passages (11%), as well as nosebleeds (8%), were reported (questionnaires supplemented with interviews). On the basis of data in Rosqvist *et al*. (49), the TPPA levels were converted to air levels. The workers were then divided into 4 exposure groups for each substance: HHPA, <1, 1 – 3, 3 – 9, and >9 μg/m^3; MHHPA, <1, 1 – 3, 3 – 15, and >15 μg/m^3. The distribution of workers with specific IgE antibodies among the 4 exposure groups was 6, 19, 25 and 29% for HHPA exposure and 24, 9, 20 and 24% for MHHPA exposure (see Table 2). The high proportion of IgE-sensitized workers in the lowest MHHPA exposure group may be explained by the fact that most of these workers were also exposed to HHPA – half of them in the highest exposure group. No such information is provided for the other exposure groups. There was a significant positive dose-response correlation between HHPA exposure and the presence of IgE and IgG antibodies, as well as eye and nose irritation (significant after adjustment for sex). No such correlation was found for MHHPA exposure, although there was a trend for induction of IgG antibodies. The authors conclude that HHPA is sensitizing (induction of IgE antibodies) down to a level of 40 fmol/ml plasma, corresponding to an air level of about 1 μg/m^3. They also address the question of whether HHPA is a more potent sensitizer than MHHPA, but conclude that this can not be determined from their study results (50). There are problems with establishing a lowest effect level on the basis of this study. The exposure was mixed, and antibodies for HHPA and MHHPA were not differentiated in the analysis. Dose-response correlations are given only for the separate anhydrides. The average MHHPA exposures in the two lowest HHPA exposure groups (<1 μg/m^3 and 1 – 3 μg/m^3) were 14 μg/m^3 (range 0.05 – 84.5) and 10.8 μg/m^3 (range 0 – 118) (calculated from original data obtained from Bo Jönsson, February 2010).

In a Japanese company that used HHPA and MHHPA as epoxy hardeners, a cross-sectional study was made several years after levels of organic acid anhydrides had been reduced to below 40 μg/m^3. Elevated levels of specific IgE were seen in 8 (25%) of 32 workers, and 5 of the 8 had work-related symptoms involving the eyes and nose. The symptoms usually appeared during operations when brief exposure peaks might be expected. The other 24 persons reported no work-related health concerns. Four of the 8 IgE-positive subjects were believed to have been sensitized by previous high exposures (68).

MTHPA. In a Swedish study of a group of 144 workers with current exposure and 26 workers with previous exposure to MTHPA, 31% had work-related eye symptoms, 53% had nasal symptoms, 26% had throat symptoms, and 11%

suffered from asthma. Corresponding figures for a control group of 33 persons from a nearby machine shop where exposure to irritating substances was low were 0, 9, 6 and 0% respectively. There were statistically proven correlations between exposure and symptoms involving eyes and upper respiratory tract, as well as dry cough. Skin prick tests with a conjugate of MTHPA and HSA were positive for 25 persons, and 28 had MTHPA-specific IgE antibodies. None of the controls had a positive prick test or specific antibodies. A total of 56 air samples were taken (50 – 300 minutes per sample), mostly with personal monitors, but stationary monitors were also used. The total sampling time was 222 hours. The concentration of MTHPA was below 150 $\mu g/m^3$. Among workers exposed to 5 – 20 $\mu g/m^3$ (n = 70), 56% had symptoms involving eyes and upper respiratory tract, 9% had asthma, and 16% had the specific antibodies. In the group (n = 55) with exposure to 20 – 150 $\mu g/m^3$, 65% had eyes and upper respiratory symptoms, 11% had asthma and 22% had the specific antibodies. Five sensitized workers who had left the company no longer had symptoms and were less reactive to methacholine, but 41 workers who had remained at the company reported no improvement in symptoms despite a tenfold reduction in exposure (42, 59).

Examinations were given to 28 workers exposed to MTHPA levels in the range 1.09 – 22.4 $\mu g/m^3$ at two Japanese companies. The air level of MTHPA was measured twice a year with stationary monitors. Specific IgE antibodies were identified in 9 (32%) of the workers, and 8 of them had upper respiratory symptoms (64). In a subsequent study of 148 workers from the same two companies, 66% were found to have specific IgE antibodies (65). In a study (probably with the same study base used in Yokota *et al.* 1997 (65) but with a different selection) of two Japanese factories (A and B) producing condensers, MTHPA levels in the assembly and inspection areas were 26 to 64 $\mu g/m^3$ (geo-metric means) with a range of 7.5 – 421 $\mu g/m^3$ in factory A and 4.9 – 5.5 $\mu g/m^3$ (range 0.7 – 22.4 $\mu g/m^3$) in factory B. MTHPA levels in air were measured with stationary monitors for 60 to 120 minutes during the afternoons, when they had reached a plateau phase. The measurements were made twice a year for two years. In factory A, 24 of 37 workers (65%) had MTHPA-specific IgE antibodies, and in factory B 38 of 58 (66%), but the sensitization might have been a consequence of previous high exposures. At both factories, work-related symptoms involving eyes and nose were significantly more common among the sensitized workers than among those not sensitized. Symptoms among sensitized persons were more common in factory A than in factory B. No cases of asthma were reported. The authors report that "…the minimal level of MTHPA that was associated with work-related symptoms was 15 – 22 $\mu g/m^3$" (66), but it is not clear how they arrived at this figure. In a later publication it is reported that, in the same study population but with an expanded study base, 96 (65%) of 147 persons were sensitized to MTHPA (specific IgE antibodies). Average exposures (geometric means) varied from 5.49 to 68.4 $\mu g/m^3$ (range 0.68 – 421). The authors point out that this high frequency of sensitization may be due to earlier high exposure levels (69).

In a German study made in 1991, examinations given to 110 workers exposed to HHPA and MTHPA. Twenty were found to be sensitized (presence of specific IgE antibodies and/or positive prick test with organic acid anhydride), and those who had work-related symptoms were further tested with inhalation provocation. The authors report that the clinical relevance of sensitization was confirmed in six of them. Air quality at the workplace was improved after the study, and the production process was modified to use only MTHPA. When the workers were again examined in 1995, air levels of MTHPA were <0.5 – 36 μg/m^3 (3 readings from personal and 5 from stationary monitors). At that time 27 of the workers examined in 1991 had left the company. The relative risk for leaving the company was 2.6 (95% CI: 1.4 – 4.9) for sensitized compared to non-sensitized workers. Ten of the 20 sensitized workers identified in the 1991 study were still at the company in 1995, including 5 of the 6 judged in 1991 to have clinically relevant sensitization. All 5 of them reported fewer symptoms, and 4 of 5 reported less severe symptoms than in 1991 (13, 14). Unfortunately, neither their exposures nor the severity of their symptoms in 1991 are clear from the report. Two more factories were included in an expanded study (15), and 7 of 20 (35%) and 29 of 113 (26%) of the workers examined were found to be sensitized (IgE antibodies specific for acid anhydride and/or positive prick test). The exposure levels for MTHPA at the two factories were 28.5 and 6.6 μg/m^3 (medians, personal monitors) but since several other acid anhydrides were also used in these factories no dose-response relationship can be established.

In a study by Nielsen *et al.* (43) a group of 43 workers were examined for immunologic markers for MTHPA exposure. Ten workers with work-related health problems and MTHPA-specific IgE had significantly higher levels of tryptase in nasal lavage fluid than 19 non-sensitized workers with work-related nasal symptoms, and also had significantly higher levels of eosinophilic cationic protein (ECP) in serum than an unexposed control group.

HHPA, **MHHPA** and **MTHPA.** In a prospective study made in three factories (62), the relation between exposure and induction of IgE antibodies was followed in 163 previously unexposed persons (66 women, 97 men). The observation time (exposure time) was 1 to 105 (average 32) months. Air levels of organic acid anhydrides (MTHPA, HHPA and MHHPA) in the three factories were measured annually with personal or stationary monitors for 9, 4 and 9 years respectively. In all 748 samples were taken, representing a total sampling time of about 2000 hours. The subjects' average exposure to HHPA and MHHPA, or to MTHPA, was 15.4 (<1 – 189) μg/m^3. Specific IgE antibodies were identified in 21 of them (13%), and the average induction time was 8.8 (range 1 – 35) months. The incidence of sensitization was 4.92 cases per 100 person-years of exposure. The proportion of sensitized subjects increased with increasing exposure: 6% at 0 – 5 μg/m^3, 10% at >5 – 10 μg/m^3, 15% at >10 – 15 μg/m^3 and 25% at >15 μg/m^3 (estimated from Figure 1 in Reference 62), which in number of persons corresponds to 2 of 34, 8 of 77, 3 of 20 and 8 of 32 (personal communication, Hans Welinder, Division of Occupational and Environmental Medicine, Lund

University, Sweden). No correlation between sensitization and peak exposure levels was seen in this study (62). Although the study contains numerous monitoring measurements, exposure peaks are still a possibility.

In the same prospective study, symptom development was followed in 146 new employees (62 women, 84 men) for up to 8.5 years (45). Air levels of organic acid anhydrides were measured annually (see above) (62). Average exposures were in the range 6 – 39 μg/m^3. The incidences of work-related symptoms per 100 person-years of exposure were 9.1 for eyes, 6.4 for nose, 4.6 for throat and 3.1 for lower respiratory tract. The conclusion drawn in this study is that exposure to organic acid anhydrides is frequently associated with dose-related symptoms involving eyes and respiratory tract (45). The authors report that symptoms also appeared at average exposures below 10 μg/m^3, but present no statistical analysis.

TCPA. Occupational asthma caused by TCPA has been verified by inhalation provocation. In several companies and several studies, workers have shown both immediate and late reactions. Positive reactions have been obtained in prick tests and RAST (radioallergosorbent tests) with TCPA-HSA conjugate. Symptoms and bronchial hyperreactivity persisted during the entire 12 years of a follow-up study made after exposure was ended by factory closure (4).

In a Canadian cross-sectional study of 52 workers in a factory where they had been exposed to TCPA for only two years, 35% had occupational asthma and 31% (15 of 49) had specific IgE antibodies. Air samples were taken with personal and stationary monitors (total 15 samples); average TCPA exposures were in the range 210 – 390 μg/m^3. There was no correlation between occurrence of specific IgE antibodies and reported work-related respiratory symptoms or FEV_1 reduction during a shift. After exposure had been reduced to <110 μg/m^3 the symptoms became less severe and there were no new cases of asthma or sensitization, but the authors point out that the follow-up time (4 months) was brief and there were only a few subjects (35).

THPA. No information on health effects caused by occupational exposure to THPA was found in the literature.

Animal data

MA and TMA have been shown to be extremely irritating to the eyes of rabbits (27). In an inhalation study, groups of rats, hamsters and monkeys were exposed to MA in concentrations of 0, 1000, 3000 or 10,000 μg/m^3 6 hours/day, 5 days/week for 6 months. Indications of irritation in eyes and nose were seen in all species at all exposure levels and were dose-related. Histopathological examinations revealed reversible inflammatory changes in the noses of all species (54). Lung function examination of the monkeys revealed no effects on lung function. Inhalation provocation with TMA of Brown Norway rats not previously sensitized had an effect on breathing pattern typical of irritation, and quite different from the pattern seen in TMA-sensitized animals (1, 2). The changes were regarded as effects on the lower rather than the upper respiratory tract. The

lowest concentration producing an effect was 14,000 μg/m^3. Similar reactions have been reported in guinea pigs (28).

Respiratory sensitization

Animals have been sensitized to PA by both inhalation and subcutaneous injection of the substance. Animals have been sensitized to TMA by inhalation, intradermal injection of TMA in oil, and skin exposure to TMA in powder form (27). Dry TMA powder applied to the skin of Brown Norway rats four times in 21 days induced TMA-specific IgE antibodies. On provocation (35 days after the initial treatment) by inhalation of a TMA aerosol in concentrations of 200 to 40,000 μg/m^3, 1000 μg/m^3 yielded an immediate allergic reaction in the form of increased airway resistance. Levels of 5000 and 40,000 μg/m^3 yielded both immediate and late reactions (76). Changes in the respiratory passages of Brown Norway rats after inhalation of TMA were also studied by Zhang *et al.* (77). The rats were exposed to aerosols of 40, 400, 4000 or 40,000 μg/m^3 for 10 minutes, once a week for 10 weeks, and then challenged with 40,000 μg/m^3. In the rats in the two highest exposure groups, the treatments resulted in development of specific IgE antibodies and both early and late allergic reactions. The reactions were strongest in the rats previously exposed to 40,000 μg/m^3 (63). Rats exposed by inhalation to TMA concentrations in the range 30 – 300 μg/m^3 had pulmonary hemorrhages and specific antibodies, whereas a level of 10 μg/m^3 had no apparent effect (29, 71).

Provocation of guinea pigs sensitized by intradermal injection of free HHPA or MTHPA resulted in both airway obstruction and plasma extravasation, and the severity of the reactions correlated to the levels of specific IgG1 antibodies in serum (74).

After guinea pigs had been sensitized by intradermal administration of equimolar amounts (0.3 M in 0.1 ml) of various organic acid anhydrides, IgG1 titers were measured (61). The result yielded the following ranking (highest titer first): MTHPA > PA = MA > TMA = HHPA = MHHPA > THPA. A similar study (75) was made of IgE antibody formation in Brown Norway rats (0.2 M in 0.1 ml), and yielded the following ranking (highest titer first): PA > TMA = HHPA = MHHPA > MA = THPA = MTHPA. The authors suggested that titers of IgG1 and IgE antibodies might be used as a measure of sensitizing potential.

Acid anhydrides, especially TMA, have also been used as model substances for respiratory sensitizers in the development of predictive animal models for testing the allergenicity of chemicals. In addition to induction of specific IgE antibodies, several immunological markers have been shown to be affected by exposure to acid anhydrides. These include the surface structure of T-cells (e.g. CD44 expression) and cytokine secretion (e.g. IL-4, IL-5, IL-10 and IL-13) (9, 10, 11, 12, 51, 56).

To summarize the animal experiments, after the animals have been sensitized, antibodies can be demonstrated against the anhydride as well as the new antigenic determinants in anhydride-modified albumin. IgG antibodies have usually been found, but in Brown Norway rats IgE antibodies have also been identified.

Provocation with either free anhydride or the corresponding albumin conjugate is followed by immediate reactions in airways, which have been registered with plethysmography. Dose-response relationships have been observed: higher exposure leads to more antibody formation and more severe toxic reactions in the form of higher bronchial reactivity, higher vascular extravasation into airways, and infiltration of eosinophilic granulocytes into the lungs. Hemorrhagic foci in lungs have been observed after exposure to TMA. Good correlations between exposure, antibody levels and lung damage exist here also. Caution should be used in extrapolating results from animal experiments to humans, since tests can produce different results in different sorts of animals. For example, different rat strains have very different reactions with regard to allergen-specific IgE. In addition, some of these studies can be difficult to reproduce because conditions, including those under which animals are kept, vary from lab to lab and may affect results.

The animal studies reviewed here confirm the findings of the epidemiologic studies, but have been conducted at exposures too high to provide any information about LOELs (Lowest Observed Effect Levels).

Mutagenicity, carcinogenicity, effects on reproduction

Ames' tests with PA and TCPA revealed no mutagenic activity. These substances had no effect on chromosome aberrations or sister chromatid exchange in either hamster ovary (CHO) cells or rat liver cells. A study with 10 mM PA, however, reports an increase of chromosome aberrations in CHO cells (27).

The International Agency for Research on Cancer (IARC) has not published a carcinogenicity classification for any of the organic acid anhydrides reviewed in this document.

Teratogenicity with exposure to PA has been studied in mice. No effects could be observed at doses that were not toxic to the mothers (27). Nor has MA shown any reproductive or teratogenic effects in animal studies (53).

Dose-effect/dose-response relationships

In the opinion of the Criteria Group, the formation of IgE antibodies specific for organic acid anhydrides can be regarded as a critical effect. This is based on the high probability that sensitized persons will develop health problems if exposure is continued. A similar stand has been taken by a Dutch expert group (20).

Dose-effect/dose-response relationships observed in humans and animals are summarized in Tables 2 and 3. The human studies were made in industries where several other chemical substances were also in use, which makes it difficult to differentiate, for example, the irritative effects of organic acid anhydrides from those of other substances. The sensitization, however, is specific. The data indicate that organic acid anhydrides are potent sensitizers.

Acute effects observed in experimental exposures of both animals and humans are irritation of mucous membranes and skin, especially with exposure to the

substances in powder form. An MA level of 1000 μg/m^3 has caused eye irritation in animals (54). Data on humans are extremely limited.

Skin exposure or intradermal injection of free or protein-bound organic acid anhydride results in dose-related immunization (formation of specific antibodies) in laboratory animals. Sensitized animals also show dose-dependent reactions to provocation (52, 74). For most of these organic acid anhydrides, it has been shown in animal studies that they are sensitizing and initiate development of IgE antibodies. This has also been observed in humans, for all the anhydrides reviewed here except THPA.

Lung hemorrhages and antibody development have been observed in rats after 2 weeks of exposure to 30 μg/m^3 TMA (Table 3) (29, 71).

Available data on human exposures has obvious gaps, and come almost entirely from cross-sectional studies. Another problem in determining the effects of specific organic acid anhydrides is the prevalence of mixed exposures. Further, there are no data on MA and THPA. One exposure factor of importance for dose-effect/dose response relationships can be whether the substance is in gas or particle form.

Dose-effect/dose-response relationships observed in studies of workers exposed to organic acid anhydrides are presented in Table 2. The correlations, grouped by substance and occurrence, are further described below.

PA and **MA**. In a cross-sectional study of workers exposed to PA, those with an average exposure of 400 μg/m^3 were likely to have work-related conjunctivitis (46%), rhinitis (40%) or asthma (14%). In the group with exposures below 100 μg/m^3 there were no cases of asthma, but 20% had conjunctivitis and/or rhinitis (40). In another study of 285 workers exposed to PA and MA there were 4 sensitized subjects with average exposure levels of 9 – 62 μg/m^3 (PA) and 2 – 3 μg/m^3 (MA) (3). There was also a low level of TMA. The study does not state which anhydrides caused the sensitization.

TMA. In a cohort of workers exposed to TMA, the prevalence of sensitization and work-related symptoms increased with exposure. Positive prick tests and work-related respiratory symptoms were significantly more frequent among workers exposed to levels above 10 μg/m^3 than among those with lower exposure (3). At higher levels (1700 – 3600 μg/m^3), 3 of 9 exposed workers developed asthma (31). In a cross-sectional study by Zeiss *et al.* (73), 2 of 8 persons in the highest exposure group (0.54 – 6500 μg/m^3, geometric mean 170 μg/m^3) had TMA-specific IgE antibodies. A study by Grammer *et al*. (18) reports development of TMA-specific IgE antibodies at an average exposure level of 2 μg TMA/m^3 (range 0.1 – 120 μg/m^3). No development of TMA-specific IgE antibodies was seen at an average exposure level of 0.51 μg TMA/m^3 (range 0.23 – 2.4 μg/m^3). The exposure measurements in this study are not fully reported, and were probably very few.

HHPA and **MHHPA.** These two substances are sensitizing at low exposure levels. In a group (n = 95) exposed to average levels up to 140 μg/m^3, 24% had

specific IgE antibodies (60). Examinations were given to 154 workers in a company using HHPA and MHHPA as hardeners in epoxy plastics (44). Air levels of organic acid anhydrides were relatively low: <1 – 94 μg/m^3 for HHPA and <3 –77 μg/m^3 for MHHPA. IgE antibodies specific for one or both of the anhydrides were identified in 22% of the subjects. Work-related symptoms were more frequent among the exposed workers than in 57 matched controls (eyes, 23% vs. 14%; nose, 28% vs. 16%; nosebleeds, 8% vs. 0%; lower respiratory tract, 10% vs. 4%). The workers were divided into three exposure categories based on current exposures (HHPA + MHHPA): <10, 10 – 50, and >50 μg/m^3. There was a signify-cant correlation between exposure and symptoms involving the eyes, nose and lower respiratory tract. Even in the lowest exposure group there was a signify-cantly higher proportion of subjects with specific IgE antibodies (13% HHPA, 15% MHHPA), although the prevalence of symptoms was not significantly elevated. In another study of the same study population the workers (n = 139) were divided into 4 exposure groups for each substance on the basis of protein adducts in plasma: HHPA <1, 1 – 3, 3 – 9 and >9 μg/m^3, and MHHPA <1, 1 – 3, 3 – 15 and >15 μg/^{3}. There was a significant positive dose-response correlation between HHPA exposure and development of IgE antibodies down to a level of about 1 μg/m^3 (50). Since it was a matter of mixed exposure and since the persons in the lowest exposure group for HHPA were also exposed to significant levels of MHHPA (14 μg/m^3) and the analysis did not differentiate between antibodies specific for the two substances, it is impossible to identify a lowest effect level in this study.

MTHPA. In a Japanese study, in a factory making condensers where average exposure was 5 μg/m^3 (range 1 – 22 μg/m^3) there was some sensitization, but it may have been a result of earlier high exposures. The prevalence of work-related symptoms involving the eyes and nose, among both sensitized and unsensitized subjects, was higher in another factory with higher average exposure, and in both factories was higher for sensitized subjects than for those not sensitized (66). Exposure levels in a German study were <0.5 –36 μg/m^3, and of 5 persons with clinically relevant sensitization, all had fewer and 4 of 5 had less severe symptoms after previously higher exposures had been reduced to these levels (13, 14). This observation is in some contrast to those made by Nielsen *et al.* (42). Among workers exposed to 5 – 20 μg/m^3 56% had symptoms involving eyes and upper respiratory tract, 9% had asthma, and 16% had specific antibodies. In a group with higher exposure (20 – 150 μg/m^3) 65% had eye and upper respiratory symptoms, 11% had asthma, and 22% had specific antibodies (42, 59).

HHPA, MHHPA and **MTHPA.** In a prospective study the relation between exposure and induction of IgE antibodies was examined in 163 previously unexposed workers (62). Observation time (exposure time) was 1 to 105 (mean 32) months. Average exposure to organic acid anhydrides was 15.4 (<1 – 189) μg/m^3. The sensitization incidence was 4.92 cases per 100 person-years of exposure. The proportion of sensitized subjects increased with increasing exposure: 6% at 0 – 5 μg/m^3, 10% at >5 – 10 μg/m^3, 15% at >10 – 15 μg/m^3,

and 25% at >15 μg/m^3 (62). In the same prospective study, symptom development was followed in 146 new employees (62 women, 84 men) for up to 8.5 years (45). The air concentrations of organic acid anhydrides (MTHPA, HHPA and MHHPA combined) were measured yearly. Exposure averages ranged from 6 to 39 μg/m^3. The incidences of work-related symptoms per 100 person-years of exposure were 9.1 for eyes; 6.4 for nose; 4.6 for throat; and 3.1 for lower respiratory tract. The authors report that symptoms also appeared at average exposures below 10 μg/m^3, but they present no statistical analysis.

TCPA. In a Canadian cross-sectional study of 52 workers in a factory where TCPA had been in use for only two years, 35% of the workers had occupational asthma and 31% (15 of 49) had TCPA-specific IgE antibodies. Exposure averages were in the range 210 – 390 μg/m^3. After exposure had been reduced to <110 μg/m^3 the symptoms became less severe and no new cases of asthma or sensitization occurred (35). The observation period was short, however, and there were few subjects.

Conclusions

The critical effect of occupational exposure to organic acid anhydrides is sensitization (development of IgE antibodies specific for organic acid anhydride). This has been observed at an average exposure of about 5 μg/m^3 to a mixture of HHPA, MHHPA and MTHPA. In another study, which has poorly described exposure estimates, sensitization to TMA was observed at an average exposure of 2 μg/m^3 TMA in air (range 0.1 – 120 μg/m^3). Sensitization can lead to allergic problems such as asthma and rhinitis. With present knowledge it is not possible to differentiate the various organic acid anhydrides regarding potency or the threshold for sensitization.

Skin exposure to both airborne and liquid organic acid anhydrides can cause contact urticaria.

Experimental animals have developed respiratory tract sensitization after dermal exposure. The amount of skin uptake, however, can not be estimated.

Table 2. Dose-effect/dose-response relationships noted in workers exposed to organic acid anhydrides via inhalation. Exposure levels are given as averages (ranges) unless otherwise specified. GM = geometric mean, Exp. int = exposure interval, TWA = 8-hour time-weighted average.

Exposure in µg/m^3 (range)	Number exposed	Average years of exposure (range)	Effects	Ref.
MA/PA/TMA[1]				
<3 (MA) 9 – 62 (PA) 0.5 – 0.9 (TMA)	285		A total of 4 cases (1.4%) with specific IgE.	3
PA				
<100	25	11.9 (0.3 – 40)	12% specific IgE 20% conjunctivitis 20% rhinitis 0% asthma	40
400	35	13.3 (0 – 43)	3% specific IgE 46% conjunctivitis 40% rhinitis 14% asthma	
TMA				
<0.53 (<0.45-<0.6) 0.51 (0.23-2.4) 2 (0.1-120) 36 (2.3-1900) 130 (2.9-1700)	24 98 79 57 28		0% TMA-specific IgE 0% TMA-specific IgE 6% TMA-specific IgE 11% TMA-specific IgE 25% TMA-specific IgE	18
<10 10 – 40 >40 (Exp.int.)	63 36 8		1 prick test positive 5 prick test positive 2 prick test positive At exposures above 10 µg/m^3 the proportions of workers with work-related respiratory symptoms and positive prick tests for TMA-specific antibodies were significantly higher than at lower exposures.	3
170 (0.54 – 6500) (GM)	8		2 had TMA-specific IgE antibodies	73
1700, 3600 (TWA)	9		11% specific IgE 44% symptoms of irritation 33% asthma	31
HHPA/MHHPA[1]				
30 (2 – 62)	32	9 (0.2 – 21)	8 (25%) had specific IgE; 5 of these had work-related eye and nasal symptoms.	68
about 35 (2 – 470)	95	7.0 (0.1 – 25)	23 (24%) subjects with specific IgE.	60

Table 2. Continue.

Exposure in µg/m^3 (range)	Number exposed	Average years of exposure (range)	Effects	Ref.
0 (controls)	57	5.0	0% specific IgE 14% eye symptoms 16% nasal symptoms 4% lower respiratory tract symptoms	44
<10	53	6.0	15% specific IgE (MHHPA) 13% specific IgE (HHPA) 15% eye symptoms 30% nasal symptoms 8% lower respiratory tract symptoms	
10 – 50	72	3.5	26% specific IgE (MHHPA) 26% specific IgE (HHPA) 25% eye symptoms 26% nasal symptoms 14% lower respiratory tract symptoms	
>50 (Exp.int.)	29	5.5	17% specific IgE (MHHPA), 21% specific IgE (HHPA) 34% eye symptoms 28% nasal symptoms 17% lower respiratory tract symptoms	
HHFA[2]				50
<1	35	4	2 (6%) HHPA/MHHPA-specific IgE	
1-3	37		7 (19%) HHPA/MHHPA-specific IgE	
3-9	32		8 (25%) HHPA/MHHPA-specific IgE	
>9 (Exp.int.)	35		10 (29%) HHPA/MHHPA-specific IgE	
MHHFA[3]				
<1	33	4	8 (24%) HHPA/MHHPA-specific IgE	
1-3	33		3 (9%) HHPA/MHHPA-specific IgE	
3-15	35		7 (20%) HHPA/MHPA-specific IgE	
>15 (Exp.int.)	38		9 (24%) HHPA/MHHPA-specific IgE	
MTHPA				
<0.5 – 36 (Exp.int.)	84		Of 5 persons with clinically relevant sensitization, all showed fewer and 4 of 5 milder symptoms after previously higher air levels had been reduced.	13, 14
5 (1 – 22) (GM)	58	12.4 (1.3 – 20)	66% specific IgE[4] 26% eye symptoms 34% nasal symptoms 0% asthma	66
26 – 64 (7 – 421) (GM)	37	5.6 (0.2 – 13)	65% specific IgE[4] 57% eye symptoms 70% nasal symptoms 0% asthma	

Table 2. Continue.

Exposure in µg/m^3 (range)	Number exposed	Average years of exposure (range)	Effects	Ref.
0 (controls)	33		0% specific IgE 9% eye/upper respiratory tract symptoms 0% asthma	42
5 – 20	70		16% specific IgE 56% eye/upper respiratory tract symptoms 9% asthma	
20 – 150 (Exp.int.)	55	2.0 (0.1 – 6.0)	22% specific IgE 65% eye/upper respiratory tract symptoms 11% asthma	
HHPA/MHHPA/ MTHPA[5]				
≤5 >5 – 10 >10 – 15 >15 (Exp.int.)	163 (total)	0.7 (0.1 – 2.9)	6% specific IgE 10% specific IgE 15% specific IgE 25% specific IgE	62
6 – 39	146	≤9.0	Incidences of work-related symptoms per 100 person-years of exposure: 9.1, eyes; 6.4, nose; 4.6, throat; 3.1 lower respiratory tract.	45
TCPA				
<110	5	<0.33	0% asthma 0% specific IgE	35
210 – 390	52	2.5 (0.1 – 8.1)	35% asthma 31% specific IgE (15/49)	

[1] Mixed exposures.
[2] Air levels calculated from protein adducts (TTPA) in plasma: <40, 40-100, >100-300 and >300 fmol TPPA from HHPA/ml. Mixed exposure with MHHPA.
[3] Air levels calculated from protein adducts in plasma: <100, 100-300, >300-1500 and >1500 fmol TPPA from MHHPA/ml. Mixed exposure with HHPA.
[4] According to the authors, the high sensitization frequency may be due to earlier high exposure levels (66).
[5] Exposure to HHPA and MHHPA, or to MTHPA.

Table 3. Dose-effect/dose-response relationships observed in laboratory animals after inhalation exposure to organic acid anhydrides.

Exposure ($\mu g/m^3$)	Number of animals	Exposure	Effects	Ref.
MA				
1000			Symptoms of irritation in eyes and upper respiratory tract.	54
TMA				
10	60	6 hrs/day, 5 days/week, 2 weeks	Limited effects on rats.	29, 71
30	60	6 hrs/day, 5 days/week, 2 weeks	Antibody production, hemorrhages in lungs.	
100	60	6 hrs/day, 5 days/week, 2 weeks	Maximum antibody production. (no higher at 300 $\mu g/m^3$)	
40	4	10 min/week, 10 weeks	0% positive IgE	77
400	4	10 min/week, 10 weeks	25% positive IgE	
4000	8	10 min/week, 10 weeks	100% positive IgE, 100% early and late asthmatic reactions on provocation.	
40,000	8	10 min/week, 10 weeks	100% positive IgE, 100% early and late asthmatic reactions on provocation.	

Potential conflicts of interest

No potential conflicts of interest have been reported.

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Summary

Montelius J (ed). Swedish Criteria Group for Occupational Standards. *Scientific Basis for Swedish Occupational Standards.* XXXI. Arbete och Hälsa 2011;45(6):1-128. University of Gothenburg, Sweden.

Critical review and evaluation of those scientific data which are relevant as a background for discussion of Swedish occupational exposure limits. This volume consists of the consensus reports given by the Criteria Group at the Swedish Work Environmental Authority from July, 2009 through September, 2010.

Key Words: Asphalt fumes, Bitumen fume, Consensus report, Formaldehyde, Occupational exposure limit (OEL), Organic Acid Anhydrides, Risk assessment, Scientific basis, Toxicology.

Sammanfattning

Montelius J (ed). Kriteriegruppen för hygieniska gränsvärden. *Vetenskapligt underlag för hygieniska gränsvärden.* XXXI. Arbete och Hälsa 2011;45(6):1-128. Göteborgs Universitet.

Sammanställningar baserade på kritisk genomgång och värdering av de vetenskapliga fakta, vilka är relevanta som underlag för fastställande av hygieniskt gränsvärde. Volymen omfattar de underlag som avgivits från Kriteriegruppen för hygieniska gränsvärden under perioden juli 2009 – september 2010.

Nyckelord: Asfaltrök, Bitumenrök, Formaldehyd, Hygieniskt gränsvärde, Organiska syraanyhdrider, Riskvärdering, Toxikologi, Vetenskapligt underlag.

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APPENDIX

Consensus reports in this and previous volumes

Substance	Consensus date	Published in Arbete och Hälsa year;volume(No)	No. in series of Consensus Reports
Acetaldehyde	February 17, 1987	1987;39	VIII
Acetamide	December 11, 1991	1992;47	XIII
Acetic acid	June15, 1988	1988;32	IX
Acetone	October 20, 1987	1988;32	IX
Acetonitrile	September 12, 1989	1991;8	XI
Acrylamide	April 17, 1991	1992;6	XII
Acrylates	December 9, 1984	1985;32	VI
Acrylonitrile	April 28, 1987	1987;39	VIII
Aliphatic amines	August 25, 1982	1983;36	IV
Aliphatic hydrocarbons, C10-C15	June 1, 1983	1983;36	IV
Aliphatic monoketons	September 5, 1990	1992;6	XII
Allyl alcohol	September 9, 1986	1987;39	VIII
Allylamine	August 25, 1982	1983;36	IV
Allyl chloride	June 6, 1989	1989;32	X
Aluminum	April 21, 1982	1982;24	III
revised	September 14, 1994	1995;19	XVI
Aluminum trifluoride	September 15, 2004	2005;17	XXVI
p-Aminoazobenzene	February 29, 1980	1981;21	I
Ammonia	April 28, 1987	1987;39	VIII
revised	October 24, 2005	2006;11	XXVII
Ammonium fluoride	September 15, 2004	2005;17	XXVI
Amylacetate	March 23, 1983	1983;36	IV
revised	June 14, 2000	2000;22	XXI
Aniline	October 26, 1988	1989;32	X
Anthraquinone	November 26, 1987	1988;32	IX
Antimony + compounds	December 8, 1999	2000;22	XXI
Arsenic, inorganic	December 9, 1980	1982;9	II
revised	February 15, 1984	1984;44	V
Arsine	October 20, 1987	1988;32	IX
Asbestos	October 21, 1981	1982;24	III
Asphalt fumes	April 14, 2010	2011;45(6)	XXXI
Barium	June 16, 1987	1987;39	VIII
revised	January 26, 1994	1994;30	XV
Benzene	March 4, 1981	1982;9	II
revised	February 24, 1988	1988;32	IX
Benzoyl peroxide	February 13, 1985	1985;32	VI
Beryllium	April 25, 1984	1984;44	V
Bitumen fumes	April 14, 2010	2011;45(6)	XXXI
Borax	October 6, 1982	1983;36	IV
Boric acid	October 6, 1982	1983;36	IV
Boron Nitride	January 27, 1993	1993;37	XIV

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

1,2-Dibromo-3-chloropropane	May 30, 1979	1981;21	I
Dichlorodifluoromethane	June 2, 1982	1982;24	III
1,2-Dichloroethane	February 29, 1980	1981;21	I
Dichloromethane	February 29, 1980	1981;21	I
Dicumyl peroxide	February 13, 1985	1985;32	VI
Dicyclopentadiene	March 23, 1994	1994;30	XV
Diesel exhaust	December 4, 2002	2003;16	XXIV
Diethanolamine	September 4, 1991	1992;47	XIII
Diethylamine	August 25, 1982	1983;36	IV
2-Diethylaminoethanol	January 25, 1995	1995;19	XVI
Diethylene glycol	September 16, 1992	1993;37	XIV
Diethyleneglycol ethylether + acetate	December 11, 1996	1997;25	XVIII
Diethyleneglycol methylether + acetate	March 13, 1996	1996;25	XVII
Diethyleneglycol monobutylether	January 25, 1995	1995;19	XVI
Diethylenetriamine	August 25, 1982	1983;36	IV
revised	January 25, 1995	1995;19	XVI
Diisocyanates	April 8, 1981	1982;9	II
revised	April 27, 1988	1988;32	IX
revised	May 30, 2001	2001;20	XXII
Diisopropylamine	February 7, 1990	1991;8	XI
N,N-Dimethylacetamide	March 23, 1994	1994;30	XV
Dimethyl adipate	December 9, 1998	1999;26	XX
Dimethylamine	December 10, 1997	1998;25	XIX
N,N-Dimethylaniline	December 12, 1989	1991;8	XI
Dimethyldisulfide	September 9, 1986	1987;39	VIII
Dimethylether	September 14, 1994	1995;19	XVI
Dimethylethylamine	June 12, 1991	1992;6	XII
Dimethylformamide	March 23, 1983	1983;36	IV
Dimethyl glutarate	December 9, 1998	1999;26	XX
Dimethylhydrazine	January 27, 1993	1993;37	XIV
Dimethyl succinate	December 9, 1998	1999;26	XX
Dimethylsulfide	September 9, 1986	1987;39	VIII
Dimethylsulfoxide, DMSO	December 11, 1991	1992;47	XIII
Dioxane	August 25, 1982	1983;36	IV
revised	March 4, 1992	1992;47	XIII
Diphenylamine	January 25, 1995	1995;19	XVI
4,4'-Diphenylmethanediisocyanate (MDI)	April 8, 1981	1982;9	II
revised	May 30, 2001	2001;20	XXII
Dipropylene glycol	May 26, 1993	1993;37	XIV
Dipropyleneglycol monomethylether	December 12, 1990	1992;6	XII
Disulfiram	October 31, 1989	1991;8	XI
Enzymes, industrial	June 5, 1996	1996;25	XVII
Ethanol	May 30, 1990	1991;8	XI
Ethanolamine	September 4, 1991	1992;47	XIII
Ethylacetate	March 28, 1990	1991;8	XI
Ethylamine	August 25, 1982	1983;36	IV
Ethylamylketone	September 5, 1990	1992;6	XII
Ethylbenzene	December 16, 1986	1987;39	VIII
Ethylchloride	December 11, 1991	1992;47	XIII
Ethylene	December 11, 1996	1997;25	XVIII
Ethylene chloride	February 29, 1980	1981;21	I
Ethylene diamine	August 25, 1982	1983;36	IV

Ethylene glycol	October 21, 1981	1982;24	III
Ethylene glycol ethylether + acetate	February 6	2009;43(4)	XXIX
Ethylene glycol methylether + acetate	June 2, 1999	1999;26	XX
Ethyleneglycol monoisopropylether	November 16, 1994	1995;19	XVI
Ethyleneglycol monopropylether + acetate	September 15, 1993	1994;30	XV
Ethylene oxide	December 9, 1981	1982;24	III
Ethylenethiourea	September 27, 2000	2001;20	XXII
Ethylether	January 27, 1993	1993;37	XIV
Ethylglycol	October 6, 1982	1983;36	IV
Ferbam	September 12, 1989	1991;8	XI
Ferric dimethyldithiocarbamate	September 12, 1989	1991;8	XI
Flour dust	December 10, 1997	1998;25	XIX
Fluorides	September 15, 2004	2005;17	XXVI
Formaldehyde	June 30, 1979	1981;21	I
revised	August 25, 1982	1983;36	IV
revised	June 9, 2010	2011;45(6)	XXXI
Formamide	December 12, 1989	1991;8	XI
Formic acid	June 15, 1988	1988;32	IX
Furfural	April 25, 1984	1984;44	V
Furfuryl alcohol	February 13, 1985	1985;32	VI
Gallium + Gallium compounds	January 25, 1995	1995;19	XVI
Glutaraldehyde	September 30, 1998	1999;26	XX
Glycol ethers	October 6, 1982	1983;36	IV
Glyoxal	September 13, 1996	1996;25	XVII
Grain dust	December 14, 1988	1989;32	X
revised	February 4, 2009	2010;44(5)	XXX
Graphite	December 10, 1997	1998;25	XIX
Halothane	April 25, 1985	1985;32	VI
2-Heptanone	September 5, 1990	1992;6	XII
3-Heptanone	September 5, 1990	1992;6	XII
Hexachloroethane	September 15, 1993	1994;30	XV
Hexamethylenediisocyanate (HDI)	April 8, 1981	1982;9	II
revised	May 30, 2001	2001;20	XXII
Hexamethylenetetramine	August 25, 1982	1983;36	IV
n-Hexanal	March 29, 2006	2006;11	XXVII
n-Hexane	January 27, 1982	1982;24	III
2-Hexanone	September 5, 1990	1992;6	XII
Hexyleneglycol	November 17, 1993	1994;30	XV
Hydrazine	May 13, 1992	1992;47	XIII
Hydrochloric acid	June 3, 2009	2010;44(5)	XXX
Hydrogen bromide	February 11, 1998	1998;25	XIX
Hydrogen cyanide	February 7, 2001	2001;20	XXII
Hydrogen fluoride	April 25, 1984	1984;44	V
revised	September 15, 2004	2005;17	XXVI
Hydrogen peroxide	April 4, 1989	1989;32	X
Hydrogen sulfide	May 4, 1983	1983;36	IV
Hydroquinone	October 21, 1989	1991;8	XI
Indium	March 23, 1994	1994;30	XV
Industrial enzymes	June 5, 1996	1996;25	XVII

Isocyanic Acid (ICA)	December 5, 2001	2002;19	XXIII
Isophorone	February 20, 1991	1992;6	XII
Isopropanol	December 9, 1981	1982;24	III
Isopropylamine	February 7, 1990	1991;8	XI
Isopropylbenzene	June 2, 1982	1982;24	III
Lactates	March 29, 1995	1995;19	XVI
Lactate esters	June 2, 1999	1999;26	XX
Laughing gas	June 7, 2006	2006;11	XXVII
Lead, inorganic	February 29, 1980	1981;21	I
revised	September 5, 1990	1992;6	XII
revised	December 8, 2004	2005;17	XXVI
Lithium and lithium compounds	June 4, 2003	2003;16	XXIV
Lithium boron nitride	January 27, 1993	1993;37	XIV
Lithium nitride	January 27, 1993	1993;37	XIV
Maleic anhydride	September 12, 1989	1991;8	XI
Manganese	February 15, 1983	1983;36	IV
revised	April 17, 1991	1992;6	XII
revised	June 4, 1997	1997;25	XVIII
Man made mineral fibers	March 4, 1981	1982;9	II
revised	December 1, 1987	1988;32	IX
Mercury, inorganic	April 25, 1984	1984;44	V
Mesityl oxide	May 4, 1983	1983;36	IV
Metal stearates, some	September 15, 1993	1994;30	XV
Methacrylates	September 12, 1984	1985;32	VI
Methanol	April 25, 1985	1985;32	VI
Methyl acetate	March 28, 1990	1991;8	XI
Methylamine	August 25, 1982	1983;36	IV
Methylamyl alcohol	March 17, 1993	1993;37	XIV
Methyl bromide	April 27, 1988	1988;32	IX
Methyl chloride	March 4, 1992	1992;47	XIII
Methyl chloroform	March 4, 1981	1982;9	II
4,4´-methylene-bis-(2-chloroaniline)	February 4, 2004	2005;7	XXV
Methylene chloride	February 29, 1980	1981;21	I
4,4'-Methylene dianiline	June 16, 1987	1987;39	VIII
revised	October 3, 2001	2002;19	XXIII
Methyl ethyl ketone	February 13, 1985	1985;32	VI
Methyl ethyl ketone peroxide	February 13, 1985	1985;32	VI
Methyl formate	December 12, 1989	1991;8	XI
Methyl glycol	October 6, 1982	1983;36	IV
Methyl iodide	June 30, 1979	1981;21	I
Methylisoamylamine	September 5, 1990	1992;6	XII
Methylisoamylketone	February 6, 2002	2002;19	XXIII
Methylisocyanate (MIC)	December 5, 2001	2002;19	XXIII
Methyl mercaptane	September 9, 1986	1987;39	VIII
Methyl methacrylate	March 17, 1993	1993;37	XIV
Methyl pyrrolidone	June 16, 1987	1987;39	VIII
α-Methylstyrene	November 1, 2000	2001;20	XXII
Methyl-t-butyl ether	November 26, 1987	1988;32	IX
revised	September 30, 1998	1999;26	XX
Mixed solvents, neurotoxicity	April 25, 1985	1985;32	VI
MOCA	February 4, 2004	2005;7	XXV

Molybdenum	October 27, 1982	1983;36	IV
revised	Februari 4, 2009	2010;44(5)	XXX
Monochloroacetic acid	February 20, 1991	1992;6	XII
Monochlorobenzene	September 16, 1993	1993;37	XIV
Monomethylhydrazine	March 4, 1992	1992;47	XIII
Mononitrotoluene	February 20, 1991	1992;6	XII
Monoterpenes	February 17, 1987	1987;39	VIII
Morpholine	December 8, 1982	1983;36	IV
revised	June 5, 1996	1996;25	XVII
Naphthalene	May 27, 1998	1998;25	XIX
Natural crystallinic fibers, except asbestos	June 12, 1991	1992;6	XII
Nickel	April 21, 1982	1982;24	III
Nicotine	June 2, 2004	2005;7	XXV
Nitric acid	June 3, 2009	2010;44(5)	XXX
Nitric oxide	December 11, 1985	1986;35	VII
revised	June 13, 2007	2008;42(6)	XXVIII
Nitroethane	April 4, 1989	1989;32	X
Nitrogen dioxide	December 11, 1985	1986;35	VII
revised	September 12, 2007	2008;42(6)	XXVIII
Nitrogen oxides	December 11, 1985	1986;35	VII
Nitroglycerin	February 13, 1985	1985;32	VI
Nitroglycol	February 13, 1985	1985;32	VI
Nitromethane	January 6, 1989	1989;32	X
Nitropropane	October 28, 1986	1987;39	VIII
2-Nitropropane	March 29, 1995	1995;19	XVI
Nitroso compounds	December 12, 1990	1992;6	XII
Nitrosomorpholine	December 8, 1982	1983;36	IV
Nitrotoluene	February 20, 1991	1992;6	XII
Nitrous oxide	December 9, 1981	1982;24	III
revised	June 7, 2006	2006;11	XXVII
Oil mist	April 8, 1981	1982;9	II
Organic acid anhydrides, some	September 12, 1989	1991;8	XI
revised	June 4, 2008	2009;43(4)	XXIX
revised	September 29, 2010	2011;45(6)	XXXI
Oxalic acid	February 24, 1988	1988;32	IX
Ozone	April 28, 1987	1987;39	VIII
revised	February 7, 2007	2008;42(6)	XXVIII
Paper dust	February 7, 1990	1991;8	XI
Penicillins	November 23, 2005	2006;11	XXVII
Pentaerythritol	November 16, 1994	1995;19	XVI
1,1,1,2,2-Pentafluoroethane	February 24, 1999	1999;26	XX
Pentyl acetate	June 14, 2000	2000;22	XXI
Peroxides, organic	February 13, 1985	1985;32	VI
Phenol	February 13, 1985	1985;32	VI
Phosphoric acid	June 3, 2009	2010;44(5)	XXX
Phosphorous chlorides	September 30, 1998	1999;26	XX
Phosphorous oxides	February 11, 1998	1998;25	XIX
Phthalates	December 8, 1982	1983;36	IV
Phthalic anhydride	September 12, 1989	1991;8	XI
Piperazine	September 12, 1984	1985;32	VI

Plastic dusts	December 16, 1986	1987;39	VIII
Platinum	June 4, 1997	1997;25	XVIII
Polyaromatic hydrocarbons	February 15, 1984	1984;44	V
Polyisocyanates	April 27, 1988	1988;32	IX
Potassium aluminium fluoride	June 4, 1997	1997;25	XVIII
Potassium cyanide	February 7, 2001	2001;20	XXII
Potassium dichromate	May 24, 2000	2000;22	XXI
Potassium Fluoride	September 15, 2004	2005;17	XXVI
Potassium hydroxide	Marsh 15, 2000	2000;22	XXI
2-Propanol	December 9, 1981	1982;24	III
Propene	September 13, 1996	1996;25	XVII
Propionic acid	November 26, 1987	1988;32	IX
Propylacetate	September 14, 1994	1995;19	XVI
Propylene glycol	June 6, 1984	1984;44	V
Propylene glycol-1,2-dinitrate	May 4, 1983	1983;36	IV
Propylene glycol monomethylether	October 28, 1986	1987;39	VIII
Propylene oxide	June 11, 1986	1986;35	VII
Pyridine	May 13, 1992	1992;47	XIII
Quartz	March 13, 1996	1996;25	XVII
Resorcinol	September 4, 1991	1992;47	XIII
Selenium	December 11, 1985	1986;35	VII
revised	February 22, 1993	1993;37	XIV
Sevoflurane	May 27, 1998	1998;25	XIX
Silica	March 13, 1996	1996;25	XVII
Silver	October 28, 1986	1987;39	VIII
Sodium cyanide	February 7, 2001	2001;20	XXII
Sodium Fluoride	September 15, 2004	2005;17	XXVI
Sodium hydroxide	August 24, 2000	2000;22	XXI
Stearates, metallic, some	September 15, 1993	1994;30	XV
Stearates, non-metallic, some	November 17, 1993	1994;30	XV
Strontium	January 26, 1994	1994;30	XV
Styrene	February 29, 1980	1981;21	I
revised	October 31, 1989	1991;8	XI
revised	April 1, 2009	2010;44(5)	XXX
Sulfur dioxide	April 25, 1985	1985;32	VI
Sulfur fluorides	March 28, 1990	1991;8	XI
Sulfuric acid	June 3, 2009	2010;44(5)	XXX
Synthetic inorganic fibers	March 4, 1981	1982;9	II
revised	December 1, 1987	1988;32	IX
revised	December 3, 2003	2005;7	XXV
Synthetic organic and inorganic fibers	May 30, 1990	1991;8	XI
Talc dust	June 12, 1991	1992;6	XII
Terpenes, mono-	February 17, 1987	1987;39	VIII
Tetrabromoethane	May 30, 1990	1991;8	XI
Tetrachloroethane	June 4, 1997	1997;25	XVIII
Tetrachloroethylene	February 29, 1980	1981;21	I
1,1,1,2-Tetrafluoroethane	March 29, 1995	1995;19	XVI
Tetrahydrofuran	October 31, 1989	1991;8	XI
Tetranitromethane	April 4, 1989	1989;32	X

Thioglycolic acid	June 1, 1994	1994;30	XV
Thiourea	December 1, 1987	1988;32	IX
revised	June 2, 1999	1999;26	XX
Thiram	October 31, 1989	1991;8	XI
Thiurams, some	October 31, 1989	1991;8	XI
Tin and inorganic tin compounds	October 22, 2003	2005;7	XXV
Titanium dioxide	February 21, 1989	1989;32	X
Toluene	February 29, 1980	1981;21	I
revised	February 6, 2002	2002;19	XXIII
Toluene-2,4-diamine	November 1, 2000	2001;20	XXII
Toluene-2,6-diamine	November 1, 2000	2001;20	XXII
Toluene-2,4-diisocyanate	April 8, 1981	1982;9	II
revised	May 30, 2001	2001;20	XXII
Toluene-2,6-diisocyanate	April 8, 1981	1982;9	II
revised	May 30, 2001	2001;20	XXII
1,1,1-Trifluoroethane	February 24, 1999	1999;26	XX
Trichlorobenzene	September 16, 1993	1993;37	XIV
1,1,1-Trichloroethane	March 4, 1981	1982;9	II
Trichloroethylene	December 14, 1979	1981;21	I
Trichlorofluoromethane	June 2, 1982	1982;24	III
1,1,2-Trichloro-1,2,2-trifluoroethane	June 2, 1982	1982;24	III
Triethanolamine	August 25, 1982	1983;36	IV
revised	October 23, 2002	2003;16	XXIV
Triethylamine	December 5, 1984	1985;32	VI
Trimellitic anhydride	September 12, 1989	1991;8	XI
Trimethylolpropane	November 16, 1994	1995;19	XVI
Trinitrotoluene	April 17, 1991	1992;6	XII
Vanadium	March 15, 1983	1983;36	IV
Vinyl acetate	June 6, 1989	1989;32	X
Vinyl toluene	December 12, 1990	1992;6	XII
White spirit	December 16, 1986	1987;39	VIII
revised	November 13, 2006	2008;42(6)	XXVIII
Wood dust	June 17, 1981	1982;9	II
revised	June 25, 2000	2000;22	XXI
Xylene	February 29, 1980	1981;21	I
revised	September 14, 2005	2005;17	XXVI
Zinc	April 21, 1982	1982;24	III
Zinc chromate	May 24, 2000	2000;22	XXI
Zinc dimethyl dithiocarbamate	September 12, 1989	1991;8	XI
Ziram	September 12, 1989	1991;8	XI

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