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Ed. Johan Montelius

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Preface

The Criteria Group for Occupational Standards at the Swedish Work Environmental Authority (SWEA) has the task of gathering and evaluating data which can be used as a scientific basis for the proposal of occupational exposure limits given by the SWEA. The Criteria Group shall not propose a numerical occupational exposure limit value but, as far as possible, give a dose-response/dose-effect relationship and the critical effect of occupational exposure.

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

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

This is the 28th volume that is published and it contains consensus reports approved by the Criteria Group during the period July, 2006 through September, 2007. These and previously published consensus reports are listed in the Appendix (p 85).

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Chairman

Johan Montelius
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Consensus Report for White Spirit

November 13, 2006

This report is based partly on an IPCS document from 1996 (31). A search of databases was made in October of 2005, and some subsequent data have also been incorporated. This report is an update of the consensus report published in 1987 (56).

In this document the term *white spirit* refers to mixtures of hydrocarbons (7 to 14 carbon atoms) with no more than 22% (by weight) aromatic hydrocarbons and <0.1% (by weight) benzene.

Chemical and physical data. Uses.

White spirits are distillates of crude oil. They are complex mixtures of straight and branched alkanes (paraffins), cycloalkanes (naphthenes) and aromatic hydrocarbons. The composition varies depending on both the crude oil used and the method of distillation. "Ordinary" white spirit (aliphatic, medium-weight white spirit) boils in the interval 150 – 215°C and usually has an aromatics content of around 15 – 20%. This type of white spirit contains hydrocarbons with 7 to 14 carbon atoms, mostly C_9 – C_{11} alkanes/cycloalkanes and C_9 – C_{10} aromatics (31, 56, 96). Dearomatized (hydrogenated) white spirit contains <1% aromatics and is predominantly C_9 – C_{12} alkanes/cycloalkanes. It has a greater proportion of cycloalkanes than ordinary white spirit: 40 – 54% (by weight) of dearomatized, medium-weight white spirit may be cycloalkanes. White spirit with very low aromatics content is sometimes called aliphatic naphtha (1, 31, 44, 57, 73, 96, 97). Since the individual components in white spirit have different vaporization rates, the composition of the gas phase above the liquid phase is not the same as that of the liquid phase, and can be expected to contain a higher proportion of lower (e.g. C_8 – C_9) hydrocarbons than the liquid phase (56, 59).

Approximate conversion factors for ordinary white spirit (boiling point interval 150 – 200°C) containing 22% (by weight) aromatics are 1 mg/m^3 = 0.17 ppm; 1 ppm = 6 mg/m^3 (1). Other types of white spirit may require other conversion factors.

White spirit at room temperature is a clear, colorless liquid with very low solubility in water and a characteristic odor. In experiments with white spirit containing about 15% aromatics (Stoddard solvent), 5 of 6 persons could detect the odor at 0.9 ppm (5 mg/m^3) and none of them at 0.09 ppm (0.5 mg/m^3) (10). In another study, the lowest concentration at which half of the subjects (n = 47) could identify the odor (Stoddard solvent) was 0.3 ppm (2 mg/m^3) (28).

Table 1. Some types of white spirit (26, 30, 31, 36, 56, 73).

Type	Synonyms	CAS No. [1]	Aromatics content (% wt.)	Vapor pressure (kPa, 20°C)	1 ppm= (mg/m^3)
Aliphatic medium wt.	White spirit type 0, Medium-weight aliphatic solvent naphtha	64742-88-7	- [2]		
Aliphatic medium wt.	White spirit type 1, Hydrogenated heavy petroleum naphtha	64742-82-1	< 25 (usually 17 – 22)		
Aliphatic	Stoddard solvent class A, White spirit, Mineral turpentine	8052-41-3	8 – 22	0.5 (25°C)	5.8[3]
Dearomatized medium wt.	White spirit type 2, Solvent-refined heavy petroleum naphtha, Medium-weight aliphatic naphtha	64741-92-0	3 – 5		
Dearomatized medium-wt.	White spirit type 3, Hydrogenated heavy petroleum naphtha, Medium-weight aliphatic naphtha	64742-48-9	< 1	0.3	6.0[4]
Dearomatized	Stoddard solvent class IIC, White spirit	64742-88-7	< 2	0.05	

[1] These CAS numbers are connected to the production process.
[2] No information available.
[3] From Reference 26 (calculated at 25°C; average molecular weight 142 g/mol).
[4] From Reference 73 (average molecular weight 143 g/mol).

White spirit is sold under a wide variety of names. Many of them are labeled with the proportion of aromatics and the boiling point (20, 31, 56). White spirit is used as a solvent and thinner for paint, enamel and asphalt products, as an extractant in the chemical industry, and as a degreaser and cleaner by mechanics, printers etc. (35, 56, 63, 73).

Uptake, biotransformation, excretion

White spirit is readily taken up via inhalation. Monitoring inhaled and exhaled air of a subject exposed while resting to 170 – 345 ppm (1000 – 2000 mg/m^3) white spirit (boiling point 150 – 200°C, 17% aromatics) for 2 hours indicated average uptake of about 50% for the aliphatic portion and 62% for the aromatics (n-decane and 1,2,4-trimethylbenzene were used as markers) (94). When subjects were exposed for 6 hours to 100 ppm (605 – 610 mg/m^3) of three different types of white spirit, it was found that uptake varied. The concentrations in venous blood

were 3.1 mg/l for white spirit containing 57% alkanes, 25% cycloalkanes and 18% aromatics (average molecular weight 145); 3.2 mg/l for white spirit with 52% alkanes and 48% cycloalkanes (average molecular weight 138), and 2.3 mg/l – significantly lower – for white spirit containing 99% alkanes and 1% cycloalkanes (average molecular weight 170) (65). In this study it was also found that with 6 hours of exposure (resting) to 50, 100 or 200 ppm (about 305, 610 or 1230 mg/m^3) of the white spirit containing 52% alkanes and 48% cycloalkanes, blood content (1.5, 3.0 and 7.2 mg/l respectively) increased with the dose (65). The deviation from a linear correlation between exposure and blood level may indicate that metabolism was saturated at the highest exposure.

There are no quantitative data on uptake of white spirit via skin or digestive tract (31). An *in vitro* study with rat skin and a similar product, kerosene (20% aromatics, mostly trimethylbenzenes, and 80% C_9 – C_{16} alkanes/cycloalkanes), showed that uptake via skin was much higher for trimethylbenzenes than for aliphatics, whereas absorption into the skin was higher for aliphatics (89). Skin uptake was also observed to be higher for aromatics (toluene, naphthalene) than for aliphatics (nonane, tridecane) in *in vitro* studies using human skin and skin from pig ears with another complex product, JP-8 jet engine fuel (18% aromatics, 82% C_8 – C_{17} aliphatics) (34). In an *in vitro* study with rat skin, uptake of JP-8 jet fuel was reported to be 0.02 mg/cm^2/hour (58). If the same rate of uptake is assumed for white spirit and human skin and the ECETOC criteria for skin notation are applied (1 hour of exposure of 2000 cm^2 skin, approximate area of hands and lower arms), the daily dose would be 40 mg. Calculating with 50% uptake and 10 m^3 inhaled air, this is equivalent to 3% of the daily dose from 8 hours of exposure to the present Swedish exposure limit of 300 mg/m^3. After uptake the various components in white spirit are distributed rapidly via blood to all the body's tissues. Due to their lipophilic nature they tend to accumulate mostly in fat tissue and brain, although their distribution patterns differ. Comparative studies with rats and C_8 – C_{10} hydrocarbons have shown that alicyclic hydrocarbons yield the highest levels in brain, whereas aromatic hydrocarbons yield much lower levels (31). The differences can probably be explained by differences in fat solubility and breakdown rate.

In a study with human subjects, the fat/blood distribution coefficient for a white spirit containing 99% alkanes and 1% cycloalkanes (average molecular weight 170) was calculated to be 47 (65, 67). In subjects exposed to 100 ppm (600 mg/m^3) of this white spirit 6 hours/day, the half time in fat tissue after redistribution was reported to be 46 to 48 hours. Terminal half times calculated from blood concentrations were 46 hours with a single 3-hour exposure and 32 hours with 5 days of exposure 6 hours/day (67). These figures should be interpreted with caution, since each component in white spirit has its own kinetics (and thus its own half time and distribution coefficient), depending on its stability and solubility in various tissues. The half times given above are approximate averages for white spirit; the actual values depend on both the composition of the product and the components analyzed. The kinetics of the individual substances in white

spirit can also be affected by the other components. Higher blood levels of 1,2,4-trimethylbenzene (1,2,4-TMB), a component of ordinary white spirit, and higher urinary excretion of the metabolite 3,4-dimethylhippuric acid were reported when exposure to white spirit was compared with exposure to pure 1,2,4-TMB (33).

There are little data on metabolism or excretion of white spirit. Studies of the individual components have shown that aliphatic hydrocarbons are oxidized to alcohol by cytochrome P450 monooxygenases in the liver. Monocyclic and polycyclic alkanes are oxidized mainly at the CH_2 groups in the ring structure, and alkylbenzene via oxidation of the alkyl portion to alcohol, and, to a lesser extent, through direct hydroxylation of the aromatic structure. This is followed, either directly or after further oxidation, by conjugation with e.g. glucuronic acid or sulfate (31).

White spirit is excreted primarily as metabolites in urine, but some of it may be eliminated via the lungs. In a study in which subjects were exposed for 7 hours to 50 or 100 ppm white spirit containing 17% aromatics, it was reported that about 12% of the exposure level of both the aliphatic and aromatic fractions was in alveolar air 10 minutes after exposure was stopped. Sixteen hours later the levels in exhaled air had dropped to 2% of the exposure level for aliphatics and 4% for aromatics (31).

Toxic effects

Human data

Acute symptoms of exposure to white spirit include irritation of eyes, nose and throat, headache, fatigue, dizziness, nausea and feelings of intoxication. Prolonged and/or heavy exposure can lead to severe brain damage, referred to as psycho-organic syndrome (POS), chronic toxic encephalopathy or solvent-related chronic encephalopathy. It is characterized by fatigue, difficulty concentrating and deterioration in memory, as well as psychological symptoms such as depression, irritability and lability. There are several epidemiologic studies associating this type of damage with exposure to various solvents, in some cases mainly white spirit, but usually a single solvent can not be identified as the primary causative factor. Further, these studies usually lack relevant exposure estimates (5, 31, 61, 64, 88, 90).

Exposure chamber studies

Eye irritation (6/6), watery eyes (3/6), throat irritation (1/6) and slight dizziness (2/6) were reported by subjects exposed for 15 minutes to 470 ppm (2700 mg/m^3) white spirt containing 14% aromatics. At 150 ppm (850 mg/m^3) one person reported slight eye irritation, and at 24 ppm (140 mg/m^3) none of them reported any symptoms. All the reported symptoms disappeared within 15 minutes after the exposure was stopped (10).

When volunteers were exposed to 700 ppm (4000 mg/m^3) white spirit containing 17% aromatics for 50 minutes (resting), there was an increase in simple

reaction time after 35 – 40 minutes (21, 94). With exposure to 110, 215, 325 or 430 ppm (625, 1250, 1875, 2500 mg/m^3) for four consecutive 30-minute sessions, however, no significant effects on the studied intellectual and psychomotor functions were observed (21).

In a study that is hard to interpret, with 6 consecutive 5-minute exposures to 103 ppm (600 mg/m^3) Stoddard solvent, an average of 7.8/25 subjects reported eye irritation, compared with 5.7/25 in controls ($p < 0.05$), and 6.8/25 reported nasal irritation vs. 3.5/25 in controls ($p < 0.01$), but no significant differences were found in records of blinking frequency, swallowing or respiratory rate (28). Hand-eye coordination tests (PPT) given immediately after the exposures revealed no effect on psychomotor function (28).

In a Danish study, 9 students (Group 1) were exposed to 0, 34, 100, 200 or 400 ppm, and 9 painters (Group 2) were exposed to 0, 50 or 100 ppm white spirit containing 17% aromatics (Varnolen™) for 7 hours (resting). Dose-related increases in upper respiratory and CNS symptoms were observed in both groups during exposure. In Group 1 there were significant increases in smarting eyes and fatigue at 200 ppm, and at 400 ppm there were also increases in nose/throat irritation, headaches and dizziness. In Group 2 there were increases in smarting eyes, nose and throat, runny noses, headache, fatigue and dizziness at 100 ppm. Respiratory rate and lung function were not affected by the exposures, and no exposure-related symptoms involving the digestive tract or peripheral nervous system were seen in either group. Clinical neurological examinations showed significant dose-related changes in walking with eyes closed and in Romberg's test in Group 1 (at 200 and 400 ppm) and in Romberg's test in Group 2 (at 100 ppm). Poorer results in neuropsychological tests of reaction time (CRT), attention (PASAT), hand-eye coordination (PPT) and memory (short-term and long-term memory with verbal learning) were also seen in the study. In Group 1, CRT and PASAT were significantly affected at 100 ppm, CRT and PPT at 200 ppm, and CRT, PASAT and PPT at 400 ppm. Further, long-term memory was also affected in Group 1 after 7 hours of exposure to 400 ppm. In Group 2 significant effects were seen only in the short-term memory test: declines in short-term memory were seen at both 50 and 100 ppm. Long-term memory tests could not be assessed in this group because the results were too poor. The reason behind the difference in sensitivity between the two groups is not clear, but age differences and prior exposure to solvents probably affected the results (14, 81).

In a Swedish study, no CNS-related symptoms (headache, fatigue, dizziness, nausea) or symptoms of irritation in eyes, nose or respiratory passages (estimated on a Visual Analogue Scale, VAS) were reported by subjects (n = 9) exposed for 2 hours in an exposure chamber (light exercise, 50 W) to 50 ppm (300 mg/m^3) white spirit containing 16% aromatics (33).

An incompletely described study reports no increase of symptoms involving the digestive tract or central/peripheral nervous system (nausea, vomiting, diarrhea, fatigue, headache, dizziness, visual disturbances, tremor, muscular weakness, ataxia, prickling in skin etc.) or dry mucosa in subjects (n = 12) after 6 hours

of exposure to 50, 100 or 200 ppm white spirit containing 52% alkanes and 48% cycloalkanes, or 100 ppm white spirit containing 57% alkanes, 25% cycloalkanes and 18% aromatics, or 99% alkanes and 1% cycloalkanes (65). These authors also report no increase in plasma concentrations of immunoglobulins (IgG, IgA, IgM) or orosomucoid (an inflammatory marker) in experimental subjects (n = 7) after exposure to 100 ppm white spirit containing 99% alkanes and 1% cycloalkanes, 6 hours/day for 5 days (66).

Occupational exposures

In a Swedish questionnaire survey, coughing and upper respiratory symptoms (nose, throat) were reported up to 2 – 3 times more frequently by persons exposed to white spirit in a workshop (n = 148) than by a reference group (n = 71) (groups further divided into smokers, former smokers, nonsmokers). The relative risk (RR) was 2.4 (95% CI: 2.0 – 2.9) for nose and throat symptoms and 1.7 (95% CI: 1.3 – 2.2) for coughing. Some subjects reported that a change had been made from "ordinary" white spirit (18% aromatics) to "dearomatized" white spirit (<1% aromatics), and some of them considered this an improvement while others thought it was worse (regarding odor and possibly skin and respiratory problems). Air concentrations were monitored in the breathing zone of the workers. A total of 34 places were monitored. The average value for all measurements was 37 ppm (215 mg/m^3). In no case did the 8-hour average exceed 85 ppm, but there were a few brief exposure peaks of up to 120 ppm. Skin exposure was also reported. Over half of those exposed to white spirit were exposed for at least 4 hours/day, and about half of them had been exposed for at least 5 years (7).

In a Finnish study, 219 house painters exposed mostly to white spirit (17% aromatics) and 229 cement workers from the same area (same age distribution) were compared. The painters reported (questionnaire) significantly higher prevalences of acute symptoms during a workday, including nausea ($p < 0.02$), feelings of intoxication ($p < 0.001$) and irritation of mucous membranes ($p < 0.05$), as well as chronic symptoms such as memory problems ($p < 0.01$), dizziness ($p < 0.02$) and decline in the sense of smell ($p < 0.001$) (31, 52, 71, 77). When the groups were tested, the group of painters had significantly worse results on 4 of 8 tests of intelligence and psychomotor performance, including tests of visual short-term memory and simple reaction time. In comparisons between a subgroup of painters up to age 40 (n = 43) and a subgroup of cement workers up to age 40 (n = 43), where previous test results from the army were considered (judged to be pre-exposure intelligence level for most of them), poorer result remained only in the visual memory test. Simple reaction time was not assessed in these sub-groups since it does not correlate to intellectual level. Time between most recent exposure and testing varied considerably from person to person (20 hours was the shortest), but this did not seem to affect the test results (52). In neurophysiological examinations (EEG, motor and sensory nerve conduction velocities) given to some of the original cohort (72 painters, 77 cement workers), the average results for the two groups were similar (77). Average exposure time for the entire group of painters

was 22 years, and their average exposure for the entire period was estimated to be 40 ppm (230 mg/m^3) white spirit per 8-hour workday. The use of water-based paints increased during the 1970s with consequent reduction of solvent-based paints, and average white spirit exposure in 1977 was reported to be 25 ppm. The (average) number of solvent-induced episodes of intoxication also declined from earlier levels during the 1974 – 1978 period. In many cases the main cause of these episodes (1960 – 1973) was reported to be solvent-based epoxy paints, which do not contain white spirit. In mapping the current exposure situation during the course of the study, it was found that there were large variations during painting (personal monitors, sampling periods 15 minutes to 3 hours). The highest concentrations of organic solvents (mostly white spirit) were measured during painting of large surfaces in small, poorly ventilated rooms such as toilets and showers. Under such circumstances, the average air concentration of white spirit during sampling was about 300 ppm (average of 11 samples) (52, 71).

Significantly worse results in 4 of 7 tests measuring intellectual functions and in 3 of 5 tests measuring psychomotor functions were reported in a cross-sectional study of workers in a rubber boot factory (n = 226), when they were compared with a control group (n = 102). When the groups were subdivided into age groups, effects on intellectual and psychomotor functions were particularly apparent in the age group 46-60: worse results on 5 of 7 intellectual and 4 of 5 psychomotor tests. These figures for the group ≤ age 30 were 2 of 7 and 2 of 5. Perception and reproduction of visual material (Bender test) and simple reaction time were particularly affected in this age group. Time elapsed between the most recent exposure and the test occasion is not reported in the study. The workers had been employed for at least 5 years and spent every 8-hour shift gluing footwear together. They used glue containing white spirit, which analyses of the glue listed as “the only toxic component”. Estimates based on measurements in the breathing zone during a shift and on company data indicated that average air concentrations during the most recent 13 years were about 85 ppm (500 mg/m^3) or a bit higher. It was also estimated that air concentrations had previously been much higher. When the workers were divided into groups according to duration of exposure, in the group with 5 – 10 years of exposure there were significant differences from controls for the Bender test, Kreapelin test (attention and mental performance), Dots location test (spatial relationships) and tests of reaction time. The most significant differences were in results for the Bender test and reaction times. Decline with increasing duration of exposure was seen for some variables (6).

In another cross-sectional study, 85 painters were compared with 85 masons. Cumulative solvent use for the painters with low exposure was ≤15 (liters/day) x years (e.g. handling ≤15 l/day for 1 year or ≤1.5 l/day for 10 years); for medium exposure 15 –30 (l/day) x years, and for high exposure >30 (l/day) x years (61). A little over half of the painters had worked only with painting buildings, and the solvent used for this was usually white spirit containing 15 – 20% aromatics. Solvents used with other types of painting usually contained more aromatic hydrocarbons (61). Subjects in the study were tested with a battery of

neuropsychological tests, including tests of intellectual and psychomotor functions and neurological tests for assessing e.g. coordination. In some cases computer tomography was also used. The odds ratios for development of dementia, corrected for age and primary intellectual level, were 1.1 (95% CI: 0.4 – 3.3) with low exposure, 3.6 (95% CI: 1.5 – 8.5) with medium exposure, and 5.0 (95% CI: 2.2– 11.4) with high exposure, but the correlations between solvent exposure and individual psychometric and neurological test results were generally weak (61). The authors concluded that a cumulative solvent exposure of up to15 (l/day) x years for painters can be considered the NOAEL (No Observed Adverse Effect Level) for organic brain damage (31, 61). This exposure measure can not be translated to a ppm level on the basis of the information given.

Neuropsychiatric effects were also examined in a Swedish study of 135 house painters and 71 carpenters with at least 10 years of work experience prior to 1971. All of them were given a battery of psychometric tests (12 tests), and information on symptoms was obtained by questionnaire and interview. The most highly exposed painters and a selection of the controls were also given neurophysiological examinations (EEG, reaction potentials, vibration thresholds, MRT). White spirit (about 17% aromatics) was the organic solvent most often used by the painters. Monitoring data from the 1970s showed that exposure levels during painting were near the limit, i.e. about 100 – 200 ppm (600 – 1200 mg/m^3) (55), which yields an average level of about 50 – 100 ppm for a workday (painting for half the time at work). The little data available from before 1970 all indicate that the threshold value then in force for white spirit exposure (1200 mg/m^3) was exceeded, sometimes by quite a bit. The painters were divided into three groups according to estimated cumulative solvent exposure (low exposure, n = 34; medium exposure, n = 67; high exposure, n = 34). Exposure episodes that led to dizziness and lightheadedness were reported by 85% of the painters and occurred in all three exposure groups. Thirty or more episodes (at least one with loss of consciousness or blackout) were reported by 26% (n = 9), 24% (n = 16), and 32% (n = 11) respectively (55). Neuropsychiatric symptoms, identified by interviews, were more common in the painters and increased with exposure. Prevalence ratios for symptoms (total index) judged by doctors to be possible indications of brain damage were 1.2 (95% CI: 0.3 – 5.5), 2.5 (95% CI: 1.0 – 6.4) and 4.5 (95% CI: 1.8 – 12) for the three groups. The number of painters who reported memory problems, irritability, sleep disturbances etc. increased with increasing cumulative exposure. For most symptoms, the risk for painters in the low-exposure group appeared to be no higher than for the controls. Digestive problems, loss of the sense of taste, and difficulty tolerating the odor of solvent were also more common among painters, and increased with exposure. Coordination tests revealed no significant differences between painters and controls (but clinical examination revealed a tendency to poorer results with increasing exposure), and very little effect was seen in the psychometric tests (55). In one test (block design) measuring visuospatial abilities, the painters had somewhat worse results ($p = 0.05$). A clear difference ($p = 0.02$) was observed when the two higher exposure

groups (combined) were compared with controls. Neurophysiological examinations revealed no significant differences between exposed subjects and controls. The authors concluded (55) that cumulative exposure to solvent concentrations <130 months (10 years) at an average level (8 hours) of about 85 ppm (500 mg/m^3) does not lead to functional and permanent effects on the nervous system, whereas exposure for 130 to 250 months (10 to 20 years) carries an increase in risk, and exposure for more than 250 months (>20 years) carries a large increase in risk for chronic toxic encephalopathy (55).

Effects on cognitive function and mental health were examined in a cross-sectional study of 110 paintmakers in two factories and 110 matched controls. The workers were exposed mostly to a mixture of white spirit, toluene, xylene, methylethylketone and methylisobutylketone. Average exposure levels were reported to be ordinarily below the occupational exposure limit. There were no significant differences between the groups with regard to prevalence of solvent-related neurotoxic symptoms or mental health (questionnaire). The subjects exposed to solvent did no worse than age-matched controls on a battery of neuropsychological tests. For the comparisons the paintmakers were divided into subgroups based on duration of exposure (range 3 – 42 years), cumulative exposure (range 12 – 1800 ppm x years) and intensity of exposure expressed as average annual 8-hour averages (range 2.6 – 60 ppm). The response frequency for the exposed group was only 42 – 43% (31, 79). Since the attrition is large and the attrition analysis only partial, no conclusions can be drawn from this study.

A relationship between human kidney damage and exposure to organic solvents, including those in paint and enamel, has been implied in some studies (9, 31). However, there are few studies of persons exposed solely or primarily to white spirit, and they provide too little data to confirm a causative relationship to white spirit. In one case, a person developed kidney failure after 1 year of cleaning floors, often up to 6 hours a day, without protective equipment. He used white spirit (Stoddard solvent) in his job, and reported that sometimes he felt “high” while he was working. A radioimmunological test to show antibodies for glomerular basal membrane was strongly positive, and a biopsy showed diffuse glomerulonephritis and focal necroses (16). There is another case report of a person who developed a syndrome with acute antibody-mediated glomerulo-nephritis (Goodpasture’s syndrome) after 5 days in a workplace where she was exposed to a “mist of mineral turpentine” (17).

A few studies have also reported liver damage and effects on bone marrow in persons exposed mostly to white spirit. No air concentrations are given in these studies, but some of them suggest that exposure levels were extremely high (31). With so little information it is hard to judge whether there is a connection to exposure. Connective tissue diseases and solvent exposure have also been discussed. A critical survey of relevant epidemiologic studies concluded that it is impossible to establish whether there is a connection between solvent exposure and any type of connective tissue disease (22). In a later study these authors state

that evidence that solvents may be a cause of systemic sclerosis is getting stronger, although no specific solvent can be identified (23).

Repeated skin contact with white spirit can lead to skin irritation and contact eczema (56).

Animal data

White spirit has low acute toxicity to experimental animals (31). The LC_{50} (8-hour) for rats exposed to ordinary white spirit containing 14% aromatics (Stoddard solvent, average molecular weight 144) is >8200 mg/m^3 (>1400 ppm) (10). For rabbits, application of 2 or 3 g white spirit/kg body weight (Stoddard solvent, 14% aromatics) on about 10% of skin for 24 hours was reported to result in hypoactivity and loss of appetite on the day after the exposure (31).

Irritation of respiratory passages, expressed as a decline of 50% or more in respiratory rate, was reported in 3 of 6 mice with 1 minute of exposure to 10,000 mg/m^3 (1700 ppm) white spirit (vapor and aerosol) containing 15% aromatics (about 11% C_9 – C_{10} alkylbenzenes). No such decline was seen with exposure to 4400 mg/m^3 (770 ppm) (10, 31). Indications of irritated mucosa were observed in rats at 400 and 800 ppm with repeated exposure to dearomatized and "ordinary" white spirit. The irritation was reported to be most pronounced in the beginning of the exposure and then gradually decline (Tables 3 and 4). Further, bronchitis-like changes (infiltration of inflammatory cells) in pulmonary tissue have been observed in several species after 90 days of constant exposure to 1271 mg/m^3 (219 ppm) white spirit containing 13 – 19% aromatics (70). In a study with rats, it is reported that histopathological changes in upper respiratory passages (infiltration of inflammatory cells, loss of cilia, hyperplasia, metaplasia) were observed at much lower exposure levels and after only 4 days of exposure (4 hours/day) to white spirit containing 19% aromatics. Vapor was generated by a sprayer. The animals were exposed to 291 mg/m^3 (50 ppm) on the first day and to about 190 mg/m^3 (about 33 ppm) thereafter. Inflammatory changes were also observed in the lungs, but were also seen in a few of the controls (72). Aerosol formation may have occurred, and the histopathological changes resemble those from aerosol exposure. The study can therefore not be used in determining a dose-effect relationship.

As to studies of the individual substances in white spirit, C_7 – C_{11} aromatics (alkylbenzenes) in mouse experiments have RD_{50} values (the concentration reducing respiratory rate by 50%) ranging from a few hundred to a few thousand ppm, and the irritation effect increases with the length of the carbon chain. The reported RD_{50} value for various trimethylbenzene isomers, for example, is 520 – 580 ppm (3, 39). With exposure to a solvent mixture containing primarily aromatic C_9 hydrocarbons (trimethylbenzene isomers, 2-, 3- and 4-ethyltoluene; total about 85%) the RD_{50} for mice was 3140 mg/m^3, somewhat higher than for pure trimethylbenzene (40). In experiments with an aerosol of a solvent based on petroleum hydrocarbons and consisting almost entirely of aromatics, especially C_9 – C_{11} hydrocarbons (about 90% alkylbenzenes, of which about 85% were C_9 –

C_{11}) there was also pronounced decrease in respiratory rate (≥50%, 6 of 6 mice) at about the same air concentration (3100 mg/m^3, 1 minute) (2, 11). The RD_{50} values for mice are much higher for n-alkanes than for the corresponding saturated alkylbenzenes: they are reported in one study to be 15,596 ppm for n-heptane, 18,155 ppm for n-octane and 62,230 ppm for n-nonane (3). In another study, the RD_{50} (mice) for n-heptane was reported to be 17,400 ppm. The RD_{50} values for n-octane, n-nonane and n-decane could not be determined in this study, but these substances were tested at lower concentrations than n-heptane (41). No RD_{50} data for the corresponding cycloalkanes were found in the literature.

Neurological effects have also been reported with exposure to white spirit. A review article (15) presents summary assessments of various parameters for neurotoxicity, based on results of numerous animal studies, and concludes that there are apparently no clear differences in neurotoxicity between white spirits with high or low aromatics content, although small differences have been reported for a few parameters and different methods/tests may have greater or lesser sensitivity (see below).

A temporary depressive effect on the central nervous system (sedation) was observed in rats with repeated exposure to 400 ppm or 800 ppm “ordinary” or dearomatized white spirit (Tables 3 and 4). In an unpublished study with rats, temporary exposure-related increases of latency time (from stimulus to response) were seen at exposures to 200, 400 and 800 ppm white spirit containing 18% aromatics, 8 hours/day for 3 days. No effect on spontaneous activity or muscular coordination was observed, however. Significantly lower nerve conduction velocity in caudal nerves of rats exposed to 800 ppm was also reported in this study (Kulig, 1989; cited in Reference 31). In another rat study, no significant exposure-related effects were seen in behavior tests measuring muscular activity, learning and memory functions at exposure to 400 or 800 ppm white spirit (20% aromatics) 6 hours/day, 5 days/week for 6 months. Muscular activity was measured before, during and after the exposure period, but the other tests were given only after an exposure-free period of 2 months (95). In a similar experiment with rats, with exposures to 400 or 800 ppm dearomatized white spirit for 6 months and testing after an exposure-free period of 2 – 3 months, reduced motor activity in darkness was noted at the higher dose level, but no other effects were seen in behavior tests measuring muscular activity, learning and memory functions (54). EEG registration of evoked potentials, however, revealed that both exposure levels affected responses to electrical stimulation of the tail (SEP), light stimulus (FEP) and sound stimulus (ABR) (54).

Neurotoxicity, measured in the tail, was examined in a study with rats. White spirit containing 0.3% aromatics (boiling point interval 150 – 200°C), 11.7% aromatics (boiling point interval 152 – 182°C), or 17% aromatics (boiling point interval 180 – 230°C) was applied to the skin of the tail (12 cm^2) 3 hours/day, 5 days/week for 6 weeks. No effect on nerve conduction velocity in motor nerves is reported, but muscle response to local electrode stimulation was affected

especially by the dearomatized white spirit. Morphological changes in skin and nerves were observed with all three substances (91).

Transmitter-related neurochemical effects and other effects indicating neuronal damage have also been demonstrated in laboratory animals after repeated inhalation exposure to white spirit (Tables 3 and 4). For some of these effects, white spirit containing 20% aromatics seems to be more potent than dearomatized white spirit. Dose-related effects on concentrations of noradrenalin (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) in the brain are reported in some studies on rats with up to 6 months of exposure to 400 or 800 ppm white spirit containing 20% aromatics, and the effects could still be seen after an exposure-free period of 2 to 4 months (43, 45, 95). These three studies (43, 45, 95) indicate that serotonergic systems are particularly affected (47). With exposure to 400 or 800 ppm dearomatized white spirit the concentrations of 5-HT and DA were significantly changed after 1 week of exposure, but not after 2 – 3 weeks of exposure (57). Effects on receptors in the serotonergic system in the brain have also been demonstrated with exposure to 800 ppm white spirit containing 20% aromatics, and to a lesser extent in experiments with dearomatized white spirit (47). Further, induction of a protein (GFAP, glial fibrillary acidic protein) that is a marker for neural damage is seen with exposure to 400 or 800 ppm white spirit containing 20% aromatics, whereas no lasting or consistent effects were observed with exposure to dearomatized white spirit (46).

Various degrees of effect on GSH and glutamine synthetase have also been shown in a few studies (8, 44, 76). In an inhalation study with rats and white spirit containing 11.7% aromatics, there was a temporary increase of GSH concentration in the cerebellum (at 4 weeks but not at 8 and 17 weeks) at 2875 mg/m^3 (500 ppm). Despite some effects on enzymes (including reduced succinate dehydrogenase activity and temporarily reduced creatine kinase activity in cerebellum, reduced creatine kinase activity in serum), 100 ppm was judged to be the NOAEL in this study (76). No effect on GSH in cerebral cortex or hippocampus was reported in another study in which rats were exposed for 3 weeks to 400 or 800 ppm white spirit containing 14 – 21% aromatics (8). Three weeks of exposure to 400 ppm dearomatized white spirit, however, yielded an increase of GSH in the brain in general, but not in the hippocampus, and at 800 ppm there was also an increase in brain (and elsewhere) of reactive oxygen species (ROS), an expression of oxidative stress (44). Glutamine synthetase in the brain was induced by white spirit containing 14 – 21% aromatics (400 or 800 ppm) but not by dearomatized white spirit at the same exposures (8, 44).

Few effects aside from irritation and CNS effects and observations related to them have been reported in experiments in which animals were exposed to white spirit in non-lethal doses. Effects on kidneys, liver and adrenal medulla (see under Carcinogenicity) have been demonstrated in some studies. Specific histopathological changes in kidneys, resultant effects on kidney function, and sometimes elevated kidney weights, have been observed in male rats after repeated inhalation exposure to white spirit (Tables 3 and 4). Such kidney changes appear because of

a special protein (α_{2u}-globulin) specific to male rats, and are not considered relevant to humans (18, 31, 82). One study reports significant increases of urea and creatinine in plasma of male rats with 6 months of exposure to 2290 or 4580 mg/m^3 (400 or 800 ppm) white spirit containing 20% aromatics (6 hours/day, 5 days/week), but histopathological examination after 4 exposure-free months revealed no exposure-related changes in kidneys (95).

Dose-dependent reductions of ALAT activity in plasma were seen in male rats exposed to 2290 or 4580 mg/m^3 (400 or 800 ppm) white spirit containing 20% aromatics, 6 hours/day, 5 days/week for 6 months. Histopathological examination 4 months after the end of the exposure revealed no exposure-related changes in livers (95). In another study with rats, increased relative and/or absolute liver weights were reported at exposure to 1970 or 5610 mg/m^3 dearomatized white spirit (<0.5% aromatics) 6 hours/day, 5 days/week for up to 3 months. No increases of ALAT or alkalic phosphatases in serum were seen in this study (69). In rats with similar exposure (138, 275, 550, 1100 or 2200 mg/m^3, 3 months) to Stoddard solvent IIC (<1% aromatics), exposure-related reduction of ALAT activity in serum was seen, on more than one occasion, at levels ≥550 mg/m^3. Significant increases in relative liver weight were seen in male rats in all exposure groups, and in the female rats absolute liver weights increased at 1100 mg/m^3. No accompanying histopathological changes in livers were observed in any exposure group. Elevated relative and/or absolute liver weights were reported in male mice at levels ≥1100 mg/m^3 (18). Two years of exposure to Stoddard solvent IIC resulted in various types of non-neoplastic and neoplastic changes in the livers of both male and female mice (see under Carcinogenicity). Increase of reactive oxygen species (ROS) in livers was reported in male rats exposed to 4679 mg/m^3 (800 ppm) dearomatized white spirit (<0.4% aromatics) 6 hours/day, 7 days/week for 3 weeks (44).

White spirit has also been tested on rabbits for skin irritation, and judged to be slightly to moderately irritating (25, 62). Unspecified white spirit, applied to the skin of guinea pigs 3 times/day for 3 days, was judged to be about as irritating as a 2% sodium lauryl sulfate solution (4). An unpublished study (cited in Reference 31) reports little or no eye irritation when 0.1 ml Stoddard solvent (14.5% aromatics) was dropped into the eyes of rabbits.

Mutagenicity, genotoxicity

White spirit containing 15% aromatics induced no mutations in *in vitro* tests with *Salmonella typhimurium* strains TA98, TA100, TA1530, TA1535, TA1537 or TA1538, either with or without metabolic activation. No significant increase of sister chromatid exchanges was seen when this white spirit was tested on human lymphocytes *in vitro* (24). Negative results were also obtained in *in vitro* tests (+/-S9) with Stoddard solvent IIC (<1% aromatics) on *Salmonella typhimurium* strains TA97, TA98, TA100 and TA1535 (63). An unpublished study (cited in Reference 31) also reports negative results when white spirit (Stoddard solvent)

containing 19% aromatics (+/- S9) was tested on *Salmonella* strains TA98, TA100, TA1535, TA1537 and TA1538, yeast, and mammalian cells *in vitro*. However, white spirit (Stoddard solvent) containing 14.5% aromatics, at doses that were more or less cytotoxic, was reported to yield positive results in *in vitro* tests on mammalian cells (unpublished study cited in Reference 31).

There are also a few *in vivo* studies. One study reports that intraperitoneal injection or inhalation exposure (50,000 mg/m^3, 5 x 5 minutes) to white spirit containing 15% aromatics did not elevate the incidence of micronuclei in bone marrow cells of mice (24). Nor did mice show evidence of chromosome damage, expressed as increase of micronuclei in red blood cells, after inhalation exposure to 138, 275, 550, 1100 or 2200 mg/m^3 Stoddard solvent IIC (<1% aromatics) 6 hours/day, 5 days/week for 3 months, and there were no indications of bone-marrow toxicity at any exposure level (63). An abstract reports that 100 or 300 ppm undefined white spirit was not mutagenic in a dominant lethal test in which rats were exposed 6 hours/day, 5 days/week for 8 weeks before mating (68). An unpublished study (cited in Reference 31) reports no mutagenic effect on gametes in a dominant lethal test with administration of Stoddard solvent to rats and mice. Further, it is reported in another unpublished study (also cited in Reference 31) that no significant increase of chromosome aberrations was seen in bone marrow of rats given Stoddard solvent (19% aromatics) by intraperitoneal injection.

Carcinogenicity

In a cancer study with inhalation exposure 6 hours/day, 5 days/week for 2 years to Stoddard solvent IIC in concentrations of 138 mg/m^3 (male rats), 550 mg/m^3 (rats, mice), 1100 mg/m^3 (rats, mice) and 2200 mg/m^3 (female rats, mice), there were effects on kidneys, adrenal medulla and liver (18). In male rats, when compared with controls, there was a higher incidence of hyperplasia in adrenal medulla at 550 mg/m^3 (23/50 vs 12/50), higher incidences of tumors in adrenal medulla (pheochromocytoma: benign or benign and malignant) at 550 mg/m^3 (13/50 vs 5/50; 13/50 vs 6/50) and 1100 mg/m^3 (17/50 vs 5/50; 19/50 vs 6/50) and elevated incidences of hyperplasia in renal tubuli and renal pelvis at 550 mg/m^3 (25/50 vs 4/50; 8/50 vs 0/50) and 1100 mg/m^3 (27/50 vs 4/50; 6/50 vs 0/50). There was also damage characteristic of renal toxicity related to chronic accumulation of α_{2u}-globulin, which is also believed to be connected to formation of renal hyperplasias and tumors (see under Animal data). In female mice there was a marginal increase of liver tumors, though this was attributed to exposure-related gain in body weight. At 2200 mg/m^3 there was a significant increase in incidence of adenomas in liver (18/50 vs 9/50). In comparison with incidences in historical controls, an increase of liver adenomas was noted in female mice at 1100 and 2200 mg/m^3 and in male mice at 550 and 2200 mg/m^3. Elevated incidences of liver adenomas/carcinomas were seen in female mice at 2200 mg/m^3 and in male mice at 550 mg/m^3. Significant increase of eosinophilic foci in liver (11/50 vs 4/50) was seen in female mice at 2200 mg/m^3, and significant increase of eosinophilic foci (14/49 vs 5/50) and basophilic foci (17/49 vs 9/50) in male mice at 1100 mg/m^3

(18). The summary carcinogenicity assessment for Stoddard solvent IIC in these studies was “some evidence” for male rats, “equivocal evidence” for female mice and “no evidence” for female rats and male mice (63).

Some epidemiologic studies have reported elevated relative risks for certain types of cancer in e.g. painters and drycleaners, who are exposed to white spirit, but there is usually also exposure to other substances (including other organic solvents), and no correlation between white spirit and cancer can be established on the basis of these studies (31).

Effects on reproduction

Human data

Little is known about risks associated with occupational exposure to solvents during pregnancy, and even less is known about possible connections between occupational exposure to solvents and effects on fertility. Solvents include aromatic hydrocarbons (e.g. toluene, xylene), aliphatic hydrocarbons (e.g. mineral spirits, kerosene), halogenated hydrocarbons (e.g. carbon tetrachloride, trichloroethylene, tetrachloroethylene) and glycol ethers. In many epidemiologic studies there is exposure to several solvents and sometimes to other types of chemicals as well. In many of the studies the exposure estimates are of poor quality. Other problems can be confounding factors and low statistical strength (31, 50, 60, 78). No epidemiologic studies of white spirit alone, which could be used to identify any effects on reproduction, were found.

However, there are a few studies that indicate elevated risk of spontaneous abortions and birth defects (including heart malformations, harelip, neural tube malformations) for occupationally exposed women, if the woman had been heavily exposed to several solvents – had reported symptoms related to solvent exposure, for example. Certain glycol ethers, toluene and tetrachloroethylene are among the individual solvents discussed as possible risk factors (31, 38, 49, 60, 78, 85).

A retrospective case-control study (85) of laboratory workers reports a significant correlation between spontaneous abortions and toluene, xylene or formalin with exposure 3 to 5 days/week during the first trimester, but not with exposure 1 or 2 days/week. Mixed exposures were common, and often included chemicals other than solvents. Eight cases had been exposed to white spirit among other substances, but the odds ratio (adjusted for factors such as smoking, alcohol consumption and previous miscarriages) for white spirit exposure (not grouped by frequency of exposure) was 1.0 (95% CI: 0.4 – 2.7). No correlation between solvent exposure and birth defects was seen in this study, but there were few cases.

Another case-control study where confounding factors were considered (including smoking, alcohol, other solvents) indicated that high exposure to aliphatic hydrocarbons, primarily of the white spirit type containing up to 15% aromatic hydrocarbons, increased the risk of spontaneous abortion in exposed women (OR = 3.9; 95% CI: 1.1 – 14.2), while low exposure yielded no significant

increase in risk. The information is based on a total of only 13 cases, 8 with high exposure and 5 with low exposure (51). A division of these cases according to type of work yielded an OR = 5.2 (95% CI: 1.3 – 20.8) for graphics work (7 cases) and OR = 2.4 (95% CI: 0.5 – 13.0) for painting (3 cases) (51). Information on air concentrations is generally lacking, but it is reported in the study that the concentration of white spirit around cleaning of printing presses had exceeded 150 ppm on 2 of 4 measurements. These workers were also exposed to several other solvents, including toluene (51).

Some indication of elevated risk for spontaneous abortion was also seen for exposed mothers in a large American case-control study with grouping according to solvent classes. Oil-based paints and paint thinners were presumed to contain aliphatic solvents, and the reported OR (adjusted for age, smoking, previous miscarriage etc.) for exposure to aliphatic solvents was 1.8 (95% CI: 1.1 – 3.0), but no clear dose-response effect was seen with division into two groups based on frequency of exposure. The OR (unadjusted) for spontaneous abortion with exposure to "paint thinners", based on 13 cases, was 2.3 (95% CI: 1.0 – 5.1), and here there was a large difference between the women who reported heavier exposure – direct skin contact, solvent odor and/or symptoms such as headache, dizziness, forgetfulness – (OR = 2.6) and the others (OR = 0.7). Elevated risk of spontaneous abortion was also seen with exposure to tetrachloroethylene and trichloroethylene. The study mentions that many of the women had mixed solvent exposures (93).

There is also some suspicion that exposure to organic solvents can affect the ability to become pregnant. Limited epidemiologic data suggest menstrual disturbances and lower fertility in women exposed to solvents, and in a few studies glycol ethers have been associated to these effects (42, 50). In a follow-up (74) to one of the studies described above (51), reduced fertility, measured as increased time to pregnancy, was reported in women with daily or high solvent exposure. When subjects were grouped by solvent type, it was noted that women with high exposure to aliphatic hydrocarbons seemed to have a somewhat higher risk for lower fertility (not significant), but 18 of the 19 women with high exposure also had high exposures to other types of solvents. In this study high exposure to halogenated hydrocarbons (including tetrachloroethylene) was also associated with reduced fertility. In a Swedish retrospective study of women exposed to various solvents and other substances in biomedical laboratories (1990 – 1994), increased time to pregnancy was related to work with solvents in general, and also to work with some individual substances including acetone (92).

As for the possibility of a connection between paternal solvent exposure and spontaneous abortion or effects on the fetus/child (birth defects etc.), epidemiologic data are inconsistent and do not provide an adequate basis for any risk assessment (13, 29, 31, 49, 83). In an older Finnish study, however, an elevated risk for spontaneous abortion was reported for wives of men with high/frequent exposure to "mixed organic solvents (including thinner)" (OR = 2.1, 95% CI: 1.1 – 3.9) and toluene; among those with elevated risk were wives of painters (OR =

3.3; 95% CI: 1.6 – 6.8). Mixed exposures were common (84). Studies focused on organic solvents and male fertility are also difficult to interpret, but some data suggest e.g. effects on sperm and endocrine functions in men exposed to solvents (including painters), or increased time to pregnancy/reduced implantation of fertile eggs in their wives. It is hard to say whether all solvents constitute a risk with high exposure or if there are some substances that are common to many solvent mixtures and that might explain the observed relationships. Certain glycol ethers, for example, are known risk factors (12, 29, 37, 42, 48, 50, 53, 75, 86, 87).

In subjects (n = 7) exposed 6 hours/day for 5 days to 100 ppm of a white spirit containing 99% alkanes and 1% cycloalkanes, there was a significant reduction of FSH concentration in serum (group average) compared with a control group (n = 5). There were only a few subjects, however, and all the values lay within the reference interval (66).

Animal data

In a study in which rats were exposed to 400 or 800 ppm white spirit (aromatics content <0.4% by weight) for 6 hours/day on days 7 – 20 of gestation, there was a slight increase of cytosol calcium concentration in synaptosomes (brain) in the pups (only female pups were examined), equally large in both exposure groups, compared with controls. The pups were killed after weaning, on day 35 – 42. No effect on body weight of the pups was observed in the study (19). In another study with similar exposure to 800 ppm dearomatized white spirit, the pups (both sexes) were tested for effects on development and behavior. Tests included neuromotor ability and muscle activity at 16 – 17 weeks, and learning/memory functions at 1 – 5 months. No significant differences were observed in either neuromotor abilities or muscle activity. Slightly poorer learning/memory functions were noted in various tests at 2 and 5 months of age. Weight gain of the mothers during the exposure period was lower, and the birth weight of the pups higher, than in controls, but no significant differences in length of gestation, number of fetuses, fetal death, sex distribution or physical development of the pups (reflexes) were noted (27).

An unpublished study (cited in Reference 31) reports elevated incidence of skeletal variations in pups, but no effect on fetal weight or litter size, in rats with exposure to 100 or 400 ppm Stoddard solvent containing 24% aromatics for 6 hours/day on days 6 to 15 of gestation. No toxic effects were seen in the mothers. An abstract (32) reports skeletal variations (higher incidence of delayed ossification, higher number of fetuses with extra ribs) and lower fetal weight in rats with maternal exposure to 950 ppm unspecified white spirit 6 hours/day on days 3 – 20 of gestation. The exposure caused poorer weight gain and eye irritation in the mothers. Another abstract (68) reports no treatment-related effects on various reproduction parameters (implantation, resorption, number of living fetuses, fetal weight, sex distribution, deformities) in the young of rats exposed to 100 or 300 ppm undefined white spirit 6 hours/day on days 6 – 15 of gestation. Higher average weight of male fetuses was reported at 100 ppm (68).

In comparison to controls, sperm motility was significantly lower in rats exposed 6 hours/day, 5 days/week for 3 months to Stoddard solvent IIC (<1% aromatics) in concentrations of 550, 1100 or 2200 mg/m^3 (77% vs 90%, 80% vs 90%, 79% vs 90%). In mice, sperm motility was significantly lower only at 2200 mg/m^3 (55% vs 61%). No significant changes in absolute weights of epididymis or testes were seen in either rats or mice, but a generally dose-related increase in relative testes weight was observed in the rats at all exposure levels (138, 275, 550, 1100, 2200 mg/m^3). No effect on the estrous cycle was seen in either rats or mice (18, 63). No exposure-related histopathological changes in testes were noted in a parallel cancer study with exposures to 138 mg/m^3 (male rats), 550 mg/m^3 (rats, mice), 1100 mg/m^3 (rats, mice) and 2200 mg/m^3 (female rats, mice) Stoddard solvent IIC, 6 hours/day, 5 days/week for 2 years (63).

Dose-effect/dose-response relationships

The most important studies with short-term exposure of volunteers and of occupational exposure to white spirit are summarized in Table 2 and below. Dose-effect relationships noted in inhalation experiments with animals and various types of white spirit are summarized in Tables 3 and 4.

Exposure chamber studies

Acute exposure to white spirit can cause irritation of mucous membranes and CNS effects in people. White spirit (14% aromatics) at 150 ppm (850 mg/m^3) caused slight eye irritation in one of six persons, and the NOAEL reported in this study was 24 ppm (140 mg/m^3) (10). A group of subjects reported eye irritation and fatigue, and had poorer results on neuropsychological and neurological tests, after exposure to 200 ppm (1160 mg/m^3) white spirit with 17% aromatics; at 100 ppm (580 mg/m^3) there were poorer results on tests of reaction time and attention, but no significant increase of symptoms. Test subjects (painters) with prior occupational exposure and considerably higher average age reported irritation symptoms (eye, nose, throat) and CNS symptoms (headache, fatigue, dizziness) and had poorer results on neurological and short-term memory tests at 100 ppm (580 mg/m^3). Short-term memory was also worse at 50 ppm (290 mg/m^3). The NOAEL in this study was 34 ppm (200 mg/m^3) (14, 81). In another study (white spirit with 16% aromatics), the reported NOAEL for irritation and CNS-related symptoms was 50 ppm (300 mg/m^3) (33). A sketchily reported study, which in some respects contradicts a previous study (14, 81), reports no increase of dry mucosa, digestive symptoms, or symptoms involving the central or peripheral nervous system in subjects exposed to 100 ppm “ordinary” white spirit (18% aromatics) or 50 – 200 ppm dearomatized white spirit (65).

Occupational exposure

There are little reliable data on air concentrations for occupational exposure to white spirit. It is therefore difficult to identify a dose-effect or dose-response

relationship from these studies. It is also difficult to determine the significance of brief, high exposure levels and mixed exposures.

In a questionnaire study, a group exposed to white spirit (average 37 ppm; 215 mg/m^3) reported coughing and upper respiratory symptoms more often than others. Although the 8-hour average never exceeded 85 ppm, there were some exposure peaks of up to 120 ppm (7). One study reports lower results in tests of intellectual and psychomotor functions in workers continuously exposed to white spirit for at least 5 years while using glue. Among the groups with worse results were those up to age 30, for whom average exposure probably did not much exceed 85 ppm (500 mg/m^3) (6). A study of house painters with cumulative exposure to solvents, primarily white spirit (about 17% aromatics), reports that an average 8-hour exposure level of about 85 ppm (500 mg/m^3) with exposure <10 years did not lead to functional or lasting effects on the nervous system, whereas exposure for 10 – 20 years was associated with increased risk, and exposure longer than 20 years with greatly increased risk of chronic toxic encephalopathy (55). A group of painters reported more frequent occurrence of chronic symptoms such as memory problems ($p<0.01$) and dizziness ($p<0.02$) , and they also had poorer results on memory and psychomotor tests. Their average exposure for the entire period (on average 22 years) was estimated to be 40 ppm (230 mg/m^3) white spirit (17% aromatics) per 8-hour workday (52, 71, 77). These data yield an average exposure of 40 ppm as the LOAEL for chronic toxic encephalopathy, provided that this syndrome is caused by exposure accumulated over a long time rather than brief exposure peaks.

Comparisons of white spirits differing in aromatics content

Although most of the comparative studies have been made with laboratory animals, the results are contradictory and no consistent differences in dose-response relationships can be given. In one study (7) of occupationally exposed subjects a change from “ordinary” white spirit (18% aromatics) to dearomatized white spirit was considered an improvement by some subjects and a detriment by others (regarding odor and possible skin and respiratory irritation). In a study (65) in which volunteers were exposed to different types of white spirit, no symptoms related to the exposures were observed.

Reductions of ≥50% in respiratory rate (RD_{50}) were noted at 3100 mg/m^3 in mice exposed to an aerosol of a crude-oil based solvent containing almost exclusively aromatics (mostly $C_9 – C_{11}$ alkylbenzenes) (11). The RD_{50} for various trimethylbenzene isomers (mice) is reported to be 520 – 580 ppm, and the reported RD_{50} exposures for n-heptane 15,596, n-octane 18,155 and n-nonane 62,230 ppm (3, 39). In another study the reported RD_{50} for n-heptane (mice) is 17,400 ppm and those for n-octane, n-nonane and n-decane could not be determined, but these were tested at lower concentrations than n-heptane (41). These studies of individual components suggest that, at these levels, aromatics are generally more irritating to mucosa than alkanes. No RD_{50} data for the corresponding cycloalkanes were found in the literature.

To address the problems arising with differences in composition and gaps in information about some of the components, a methodology has been proposed for calculating occupational exposure limits for crude-oil based solvents of the hydrocarbon type (59). This method of calculation is said to be applicable to solvents that contain aliphatic, alicyclic and aromatic hydrocarbons (mostly C_5 – C_{15}) and have a boiling point in the interval 35 – 320°C. In this method, the percentages of the various component groups are divided by applicable "guidance values" and then added. These guidance values are set for groups of substances with similar structure and similar physical, chemical, and (as far as is known) toxicological characteristics, and are based on the assumption of an additive effect. Against the background of American (ACGIH), German, British and EU exposure limits, a proposed guidance value of 200 mg/m^3 is set for C_7 – C_8 aromatics and 100 mg/m^3 for C_9 – C_{15} aromatics (with the exception of naphthalene) to prevent irritation of eyes and respiratory passages and acute CNS effects. In the same way, a guidance value of 1500 mg/m^3 is set for C_5 – C_8 aliphatics/cycloaliphatics (except for n-hexane) to prevent acute CNS effects and irritation of mucosa, and 1200 mg/m^3 is set for C_9 – C_{15} hydrocarbons of the aliphatic/cycloaliphatic type (primarily to prevent acute CNS effects). A threshold limit for white spirit calculated by the above method is much higher for dearomatized white spirit than for white spirit containing 15 – 20% aromatics. In both cases the exposure limit is somewhat lower if it is based on the composition of the liquid phase rather than the vapor phase (59). It should be noted that several endpoints (primarily acute CNS effects and irritation of mucosa) were used in deriving these guidance values, that exposure limits for the individual components can vary from country to country (and be both higher and lower than the group values given above) and that there are no exposure limits at all for many of the components. Further, these guidance values were calculated on the assumption that all interactions between the components are additive.

Conclusions

The critical effects of occupational exposure to white spirit are judged to be effects on the central nervous system and irritation of mucous membranes. In exposure chamber studies with subjects having a history of occupational exposure, there were acute CNS effects at 50 ppm and irritation complaints at 100 ppm. Irritation has been reported with occupational exposure at an average level of 37 ppm (exposure peaks up to 120 ppm). Chronic toxic encephalopathy has been reported in painters at an average exposure of 40 ppm for 22 years. They had also been exposed to other solvents and high exposure peaks.

Skin contact with liquid white spirit can remove natural oils and cause skin irritation and contact eczema.

Available studies shed no light on the relevance of aromatics content to the CNS effects. Animal studies with high exposure levels are not in clear agreement, but indicate that white spirit with high aromatics content may be more irritating to mucosa than white spirit with low aromatics content.

Table 2. Effects of short-term experimental exposure or occupational exposure to white spirit.

Aromatics content	Number of subjects	Exposure mg/m^3 (ppm)	Effects	Ref.
17%	8	4000 (700) 50 minutes	Prolonged reaction time.	21, 94
14% (v/v)	6	2700 (470) 15 minutes	Eye irritation (6/6), bloodshot eyes (2/6), watery eyes (3/6), throat irritation (1/6), slight dizziness (2/6).	10
17%	9	2320 (400) 7 hours	Smarting eyes, nose, throat; fatigue, headache, dizziness. Worse results on neuropsychological tests and in neurological examination.	14, 81
17%	14	625 (110) + 1250 (215) + 1875 (325) + 2500 (430) 4 x 30 min.	No significant effects on tests of intellectual and psychomotor functions.	21, 94
0%	12	1228 (205) 6 hours	NOAEL in this study. (dry mucosa, symptoms involving digestive tract or central/peripheral nervous system)	65
17%	9	1160 (200) 7 hours	Smarting eyes, fatigue, worse results on neuropsychological tests and in neurological examination.	14, 81
14% (v/v)	6	850 (150) 15 minutes	Slight eye irritation (1/6).	10
17%	9 students (Group 1) 9 painters (Group 2)	580 (100) 7 hours	Group 2: CNS symptoms, irritation, worse results in neurological examination. Groups 1 and 2: Worse results on neuro-psychological tests.	14, 81
18%	12	610 (100) 6 hours	NOAEL in this study. (dry mucosa, symptoms involving digestive tract or central/peripheral nervous system)	65
Not given	226 exposed 102 controls	500 (85)[1] occupational exposure	Exposed group had poorer results on tests of intellectual and psychomotor functions.	6
about 17%	135 exposed 71 controls	300-600 (50-100)[2] occupational exposure	Cumulative exposure to 85 ppm (8 hours/day) yields at <10 years no lasting functional effects on the nervous system; at 10-20 years yields increased risk; at >20 years greatly increased risk of chronic toxic encephalopathy.	55

Table 2. Cont.

Aromatics content	Number of subjects	Exposure mg/m^3 (ppm)	Effects	Ref.
16%	9	300 (50) 2 hours	NOAEL in this study. (irritation and CNS symptoms)	33
17%	9 painters	290 (50) 7 hours	No irritation or CNS symptoms. Poorer results on short-term memory tests	14, 81
17%	219 exposed 229 controls	230 (40)[3] occupational exposure	Higher prevalence of irritation and CNS symptoms; worse results on neuro-psychological tests including visual short-term memory, simple reaction time.	52, 71, 77
18% <1%	148 exposed 71 controls	215 (37)[4] occupational exposure	Coughing and upper respiratory symptoms were more common in the persons exposed to white spirit.	7
17%	9	200 (34) 7 hours	NOAEL in this study. (irritation, CNS-related symptoms, neuropsychological tests, neurological examination)	14, 81
14% (v/v)	6	140 (24) 15 minutes	NOAEL in this study. (irritation, CNS symptoms)	10

[1] Average level for 13 years (considerably higher earlier); at least 5 years of exposure.
[2] Average values during a workday; on average 100-200 ppm during painting (about 50% of the time) (200 ppm considerably exceeded prior to 1970, i.e. for 9 or more years).
[3] Lifetime average: average exposure time 22 years, average concentrations up to 300 ppm during painting (sampling time 15 minutes to 3 hours).
[4] Average value for 34 measurements; in no case did the average 8-hour value exceed 85 ppm, but there were brief exposure peaks up to 120 ppm.

Table 3. Dose-effect relationships observed in some studies of animals exposed by inhalation to white spirit containing 12 – 21% aromatics.

Aromatics content	Exposure mg/m^3 (ppm)	Species (sex)	Effects	Ref.
14% (v/v)	4600 (800) 8 hours	Rat (males)	Irritation of eyes and nose.	10
20% (v/v)	4580 (800) 6 hours/day, 5 days/week, 6 months + 4 exposure-free months	Rat (males)	Brain: relative increase of intrasynaptosomal NA, DA, 5-HT; absolute increase of intrasynaptosomal NA, DA; other changes.	45
20% (v/v)	4580 (800) 6 hours/day, 5 days/week, 6 months + 2-4 exposure-free months	Rat (males)	Indications of sedation and irritated mucosa (especially in the beginning); no effects on behavior tests. Brain: increase of 5-HT in some regions, decrease of 5-HT in cerebellum, increase of NA in e.g. hippocampus, increase of DA in e.g. thalamus, decrease of DA in hippocampus. Plasma: increase of urea and creatinine, reduction of ALAT. No exposure-related changes in histopathological examination of liver, kidneys, adrenals, heart, spleen, testes, brain.	95
14 - 21% (v/v)	4580 (800) 6 hours/day, 7 days/week, 3 weeks	Rat (males)	Indications of sedation, irritated mucosa (especially week 1). Brain: increase of glutamine synthetase activity in hippocampus. Liver and kidneys: reduction of GSH and glutamine synthetase activity.	8
20% (v/v)	4580 (800) 6 hours/day, 7 days/week, 3 weeks	Rat (males)	Indications of sedation and irritated mucosa (especially week 1). Brain: no effects on examined markers for cell damage (N-acetyl aspartate, creatine, phosphocreatine, choline-containing components, lactate).	80
20% (v/v)	4581 (800) 6 hours/day, 7 days/week, 3 weeks	Rat (males)	Brain: increase of GFAP in some regions including cerebellum and medulla oblongata.	46
20% (v/v)	4581 (800) 6 hours/day, 7 days/week, 3 weeks	Rat (males)	Indications of sedation, irritated mucosa (especially week 1). Brain: effects on synaptosomal marker proteins and receptors in serotonergic system (5-HT_{2A} receptor: reduced B_{max}, increased receptor affinity; 5-HT_4 receptor: increased receptor affinity); other changes.	47
20% (v/v)	4580 (800) 6 hours/day, 5 days/week, 3 weeks	Rat (males)	Brain: relative increase of intrasynaptosomal NA, DA, 5-HT, absolute increase of intrasynaptosomal DA, other changes.	45
20% (v/v)	4580 (800) 6 hours/day, 5 days/week, 3 weeks	Rat (males)	Brain: increase of DA, 5-HT, NA.	43

Table 3. Cont.

Aromatics content	Exposure mg/m^3 (ppm)	Species (sex)	Effects	Ref.
11.7% (w/w)	2875 (500) 6 hours/day, 5 days/week, 4 – 17 weeks	Rat (males)	Cerebellum: reduced succinate dehydrogenase activity, reduced and then increased creatine kinase activity, temporary increase of GSH, other changes. Serum: reduced creatine kinase activity.	76
20% (v/v)	2290 (400) 6 hours/day, 5 days week, 6 months + 4 exposure-free months	Rat (males)	Brain: relative increase of intrasynaptosomal DA, 5-HT, NA; absolute increase of intrasynaptosomal NA, 5-HT; other changes.	45
20% (v/v)	2290 (400) 6 hours/day, 5 days/week, 6 months + 2-4 exposure-free months	Rat (males)	Indications of sedation and irritated mucosa (especially in the beginning); no effects on behavior tests. Brain: increase of NA and DA, reduction of 5-HT and NA in cerebellum. Plasma: increase of urea and creatinine, reduction of ALAT. No exposure-related changes in histo-pathological examination of liver, kidneys, adrenals, heart, spleen, testes, brain.	95
20% (v/v)	2290 (400) 6 hours/day, 5 days/week, 3 weeks	Rat (males)	Brain: relative increase of intrasynaptosomal DA, 5-HT, NA; other changes.	45
20% (v/v)	2290 (400) 6 hours/day, 7 days/week, 3 weeks	Rat (males)	Brain: increase of GFAP in cerebellum and medulla oblongata.	46
14-21% (v/v)	2290 (400) 6 hours/day, 7 days/week, 3 weeks	Rat (males)	Indications of sedation and irritated mucosa (especially week 1). Brain: increase of GS activity in hippocampus. Liver and kidneys: reduction of GSH and glutamine synthetase activity.	8
20% (v/v)	2290 (400) 6 hours/day, 5 days/week, 3 weeks	Rat (males)	Brain: increase of NA.	43
14% (v/v)	1900 (330) 6 hours/day, 5 days/week, 13 weeks	Rat (males) Dog (males)	Rats: histopathological changes in kidneys[1], elevated BUN[2]. Dogs: no effects observed in hematological, clinical-chemical, histopathological examinations.	10
13-19%	1353 (230) 8 hours/day, 5 days/week, 6 weeks	Rat Rabbit Guinea pig Monkey Dog	All species: no significant hematological changes, no consistent histopathological changes other than possible indications of irritation in lungs of guinea pigs.	70

NA = noradrenalin; DA = dopamine; 5-HT = 5-hydroxytryptamine; GFAP = glial fibrillary acidic protein; ALAT = alanine aminotransferase; GSH = glutathione

[1] Histopathological changes specific to male rats and not relevant to humans.

[2] Probably related to the histopathological changes in kidneys.

Table 4. Dose-effect relationships in some studies of animals exposed by inhalation to dearomatized white spirit.

Aromatics content	Exposure mg/m^3 (ppm)	Species (sex)	Effects	Ref.
<0.5%	5610 (890) 6 hours/day, 5 days/week, 4, 8, 12 weeks	Rat (males, females)	Both sexes: no increase of ALAT or alkalic phosphatases in serum; elevated relative and/or absolute liver weight; no effects in hematological examination. Females: no exposure-related histopathological changes. Males: poorer weight gain; elevated relative and/or absolute kidney weight; histopathological changes in kidneys[1].	69
<0.4% (w/w)	4679 (800) 6 hours/day, 5 days/week, 6 months + 2 – 6 months exposure-free	Rat (males)	Indications of sedation and irritated mucosa (especially weeks 1-2); somewhat lower muscle activity during active periods, no other effects in behavior tests; effects on brain waves with somatosensory, light and sound stimuli, no histopathological changes in liver, kidneys, adrenals, heart, spleen, testes, nerves.	54
<0.4% (w/w)	4679 (800) 6 hours/day, 7 days/week, 3 weeks	Rat (males)	Indications of sedation and irritated mucosa (especially week 1); elevated kidney weight. Brain: increase of ROS (hippocampus) and GSH. Liver: increase of ROS, reduced glutamine synthetase activity. Kidneys: reduction of ROS.	44
<0.4% (w/w)	4679 (800) 6 hours/day, 7 days/week, 3 weeks	Rat (males)	Indications of sedation and irritated mucosa (especially week 1). Brain: effects on receptors in serotonergic system (5-HT_{2A} receptor: reduced B_{max}), no effects on synaptosomal marker proteins.	47
<0.4% (w/w)	4679 (800) 6 hours/day, 7 days/week, 1, 2, 4 weeks	Rat (males)	Brain: increase of GFAP in cerebellum after 1-2 weeks (not 4 weeks); no consistent effects on GFAP in other regions.	46
<0.4% (w/w)	4679 (800) 6 hours/day, days 7-20 of gestation	Rat (females)	Brain: slight increase of cytosol calcium concentration in synaptosomes of pups.	19
<0.4% (w/w)	4679 (800) 6 hours/day, days 7-20 of gestation	Rat (females)	Slightly worse learning/memory functions in pups at 2 and 5 months; no significant differences in neuro-muscular performance or muscle activity at 16-17 weeks. Lower weight gain in mothers, higher birth weights (pups); no significant differences in length of gestation, number of young, fetal mortality, sex distribution or physical development of pups (reflexes).	27
<0.4%	4580 (800) 6 hours/day, 5 days/week, 3 weeks	Rat (males)	Indications of sedation and irritated mucosa (especially week 1). Brain: temporary increase of DA, temporary reduction of 5-HT.	57

Table 4. Cont.

Aromatics content	Exposure mg/m^3 (ppm)	Species (sex)	Effects	Ref.
<0.4% (w/w)	2339 (400) 6 hours/day, 5 days/week, 6 months + 2 – 6 months exposure-free	Rat (males)	Indications of sedation and irritated mucosa (especially weeks 1-2); no effects on behavior tests; effects on brain waves with somatosensory, light and sound stimulation; no histopathological changes in liver, kidneys, adrenals, heart, spleen, testes, nerves.	54
<0.4% (w/w)	2339 (400) 6 hours/day, 7 days/week, 3 weeks	Rat (males)	Indications of sedation and irritated mucosa (especially week 1); elevated kidney weight. Brain: increase of GSH.	44
<0.4% (w/w)	2339 (400) 6 hours/day, 7 days/week, 1, 2, 4 weeks	Rat (males)	Brain: increase of GFAP in cerebellum after 1-2 weeks (not 4 weeks); no consistent effects on GFAP in other regions.	46
<0.4% (w/w)	2339 (400) 6 hours/day, days 7-20 of gestation	Rat (females)	Brain: slight increase of cytosol calcium concentration in synaptosomes of pups.	19
<0.4%	2290 (400) 6 hours/day, 5 days/week, 3 weeks	Rat (males)	Indications of sedation and irritated mucosa (especially week 1). Brain: temporary reduction in 5-HT.	57
<1%	2210 (400) 6 hours/day, 5 days/week, 2 years	Rat (females) Mouse (males, females)	Female rats: lower survival; no effects in histopathological examination. Male mice: no significant increase of liver tumors. Female mice: higher body weight, significant increase of eosinophilic foci, significant increase of liver tumors.	18, 63
<1%	2220 (400) 6 hours/day, 5 days/week, 3 months	Rat (males, females) Mouse (males, females)	Rats (both sexes): reduction of ALAT. Male rats: elevated relative kidney, liver and testes weights, elevated absolute kidney weight, histo-pathological changes in kidneys[1], lower sperm motility. Mice (both sexes): no effects in hematological or histopathological examination. Male mice: elevated absolute and relative liver weights, lower sperm motility.	18, 63
<0.5%	1970 (312) 6 hours/day, 5 days/week, 4, 8, 12 weeks	Rat (males, females)	Both sexes: no increase of ALAT or alkalic phosphatases in serum. Female rats: elevated absolute liver weight (week 12). Male rats: elevated relative kidney weight (week 8), histopathological changes in kidneys[1].	69

Table 4. Cont.

Aromatics content	Exposure mg/m^3 (ppm)	Species (sex)	Effects	Ref.
<1%	1110 (200) 6 hours/day, 5 days/week, 2 years	Rat (males, females) Mouse (males, females)	Male rats: lower survival, elevated incidence of pheochromocytomas in adrenal medulla, elevated incidence of hyperplasia in kidneys, increase of chronic nephropathy[2]. Female rats: no effects on histological examination. Male mice: significant increase of eosinophilic and basophilic foci. Female mice: higher body weight, non-significant increase in liver tumors.	18, 63
<1%	1110 (200) 6 hours/day, 5 days/week, 3 months	Rat (males, females) Mouse (males, females)	Rats (both sexes): reduction of ALAT. Male rats: elevated relative kidney, liver and testes weights, elevated absolute kidney weight, histo-pathological changes in kidneys[1], lower sperm motility. Female rats: elevated absolute kidney and liver weights. Mice (both sexes): no effects in hematological or histopathological examination. Male mice: elevated relative liver weight.	18, 63
<1%	550 (100) 6 hours/day, 5 days/week, 2 years	Rat (males, females) Mouse (males, females)	Male rats: elevated incidences of hyperplasia and pheochromocytoma in adrenal medulla, elevated incidence of hyperplasia in kidneys, increase of chronic nephropathy[2] Female rats: no effects in histological examination. Male mice: non-significant increase in liver tumors. Female mice: higher body weight.	18, 63
<1%	550 (100) 6 hours/day, 5 days/week, 3 months	Rat (males, females) Mouse (males, females)	Rats (both sexes): reduction of ALAT. Male rats: elevated relative kidney, liver and testes weights, elevated absolute kidney weight, histo-pathological changes in kidneys[1], lower sperm motility. Mice (both sexes): no effects in hematological or histopathological examination.	18, 63
<1%	138 (25) 6 hours/day 5 days/week, 2 years	Rat (males)	Lower survival, increase of chronic nephropathy[2]	18, 63
<1%	138 (25) 6 hours/day, 5 days/week, 3 months	Rat (males, females) Mouse (males, females)	Male rats: elevated relative kidney, liver and testes weights. Female rats: temporary reduction of ALAT. Mice (both sexes): no effects in hematological or histopathological examination.	18, 63

DA = dopamine; 5-HT = 5-hydroxytryptamine; GFAP = glial fibrillary acidic protein; ALAT = alanine aminotransferase; GSH = glutathione; ROS = reactive oxygen species

[1] Histopathological changes that are specific to male rats and not relevant to humans.

[2] Common spontaneous syndrome in male rats of this strain.

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Consensus Report for Ozone

February 7, 2007

This document is an update of the Consensus Report published in 1987 (83), and is based on original articles registered in databases through March, 2006.

Chemical and physical data

CAS No:	10028-15-6
Formula:	O_3
Molecular weight:	48
Boiling point:	- 111.3°C
Melting point:	- 192.5°C
Density:	1.96 g/l (25°C, 101.3 kPa)
Conversion factors:	1 ppm = 2 mg/m^3
	1 mg/m^3 = 0.5 ppm

Ozone at room temperature is a bluish, irritating and corrosive gas with a characteristic odor (noticeable around electrical discharges). Nine of ten subjects can smell ozone at 0.02 ppm (50). At 0°C, at most 0.49 ml ozone will dissolve in 100 ml water. Ozone breaks down to oxygen. The half time at room temperature is about 30 minutes, but is affected by the amount of pollutants in the air – the more pollutants, the shorter the half time. Ozone is a strong oxidant, and at high concentrations it is explosive.

Occurrence, use

Ozone is formed by electrical discharges or photochemical reactions in ultraviolet radiation (UV light, wavelength 185 – 210 nm), and is found in varying concentrations throughout the atmosphere. Ozone can be formed in the presence of UV light and pollutants, especially nitrogen dioxide (NO_2) and volatile organic compounds (VOC) (65). Factors that affect local ozone concentration near ground level include temperature, solar radiation and elevation. Ozone levels are generally higher in summer than in winter. In Sweden, the average winter ozone levels (monthly means) are around 0.025 ppm (Swedish Environmental Research Institute Ltd 2004/2005, www.ivl.se/miljo/projekt/ozon/), whereas 8-hour average during spring and summer can exceed 0.06 ppm, with a few readings approaching 0.1 ppm (City of Stockholm Environmental and Health Administration, SLB-analys 2005; www.slb.mf.stockholm.se/slb). Concentrations of ozone in areas with vehicular traffic are as a rule extremely low, since in the presence of the

excess NO from engine exhaust the ozone rapidly breaks down in forming NO_2 and O_2.

When air passes from the outdoor to the indoor environment ozone levels drop sharply, since the ozone binds to various surfaces (e.g. in ventilation equipment). In a study made in the U.S., for example, indoor levels were estimated to be about 20% of those outdoors (116).

Here in Sweden, the Swedish Environmental Protection Agency is responsible for seeing that environmental quality standards are followed, by monitoring where ozone concentrations are highest. In the United States, the Environmental Protection Agency (EPA) makes running compilations of air quality standards for ground-level ozone (http://www.epa.gov/ttn/naaqs/standards/ozone/s_o3_cr_td.html).

In one study, ozone exposure was measured for 115 welders of mild steel. Three whole-day measurements were made for each subject. Six of 306 readings were above 0.1 ppm, and a further 18 were above 0.05 ppm. The highest single measurement was 0.33 ppm (detection limit is about 0.005 ppm). The welding methods used were mostly MIG and MAG, but some subjects did TIG, rod or powder welding (personal communication, 2005, Håkan Tinnberg, Department of Occupational and Environmental Medicine, University Hospital, Lund). Ozone concentrations are generally higher around gas-shielded welding in stainless steel and aluminum (72, 123).

Concentrations of ozone in cabin air during high-altitude commercial flights were previously up to 0.4 ppm (106, 118). These concentrations are not comparable to the present average of 0.01 ppm and maximum level of 0.04 ppm, measured in a later study where fresh air flowing into the cabin was passed through an ozone converter (80).

In Sweden, ozone has been used to bleach paper pulp since 1993 (120). Since local concentrations vary greatly, it is difficult to determine the amount of ozone an individual worker is exposed to. Very high exposures – above 10 ppm – have occurred in accidents. Stationary monitors normally show low ozone levels (below 0.04 ppm) with several exposure peaks. In a mixer room at a pulp mill, levels above 0.05 ppm were measured 19 days per month, and levels above 0.3 ppm 10 days per month (97, 98).

Occupational exposure to ozone can also occur in connection with producing certain chemicals, in oil refineries and in the plastics industry (39), as well as around sterilization of surgical equipment, photocopying (134), water purification, and deodorization of air (4, 117) and water.

Exposure levels in various occupations are given in the section “Studies of exposed workers”.

Determining ozone in air

Ozone in workplace air can be monitored with either stationary or personal monitors. Personal monitors are preferred for constant or frequent contact with ozone. Workplace air can contain a complex mixture of reactive substances (e.g.

around welding), although some work environments (e.g. pulp bleacheries) provide a more "pure" ozone exposure. The presence of other air contaminants that react with ozone makes it difficult to generalize from workplace to workplace, even though the jobs may be similar.

Ozone in outdoor air can be measured with stationary monitors, usually placed a couple of meters above the ground or on a roof. Factors such as reactions with other substances can lead to discrepancies between roof-level and street-level measurements.

Ground-level ozone is formed by chemical reactions between nitrogen oxides and volatile organic compounds (VOC). The reactions are accelerated by sunlight and high temperatures. Ozone levels can vary greatly with weather conditions, season, time of day and changes in the amount of ozone brought in from remote locations by air movement. In Sweden, the dominant source of environmental ozone is air from central Europe. In warmer countries with high VOC emissions and high solar radiation, the dominant sources are local. Ozone can be broken down by the nitric oxide in vehicle exhausts. Ozone levels in Sweden, therefore, are lower in cities than in more sparsely populated areas. When high-pressure zones with weak breezes have remained over central Europe for a long time, the air over the continent can become quite polluted. When the pollutants are carried up to Sweden, the result is an "ozone episode" – for two or three days ozone levels are double or triple their normal values. Although these episodes seem to have become less frequent in recent years, the amount of ozone in Swedish metropolitan areas is apparently increasing (source: http://www.naturvardsverket.se/en/In-English/Menu/).

Uptake, biotransformation, excretion

Analyses of inhaled and exhaled air show that about 90% of inhaled ozone is absorbed in the respiratory passages – much of it (about 30 – 40%) in the nose and mouth. The ozone molecules react rapidly with components in the moisture coating the respiratory epithelium, forming reactive metabolites (reactive oxygen species, ROS) (see also below under "Toxic effects"). Unaltered ozone has thus little chance of reaching the outer layer of cells in the respiratory passages, and it is even more unlikely that ozone reaches the circulatory system (31, 109).

Ozone and water can form metabolites such as hydrogen peroxide (31). The half time of ozone in water is 5 to 20 minutes, depending on the pH of the water.

Toxic effects

The toxic effects of ozone are attributed to the formation of free radicals, which may react, for example, with sulfhydryl groups in proteins, or break down unsaturated fatty acids (8, 33, 92, 104). With ozone-induced cell damage, arachidonic acid is liberated and forms prostaglandins in the respiratory passages, which by various mechanisms can cause inflammation and bronchial hyperreactivity (10, 39, 112). Exposure to environmentally hazardous oxidative gases,

including ozone, leads to oxidative changes in proteins and peptides in the lungs (66, 94, 109).

Respiratory passages

Studies with volunteers

Reduction of lung function – forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) – as well as symptoms such as coughing, chest pain, breathlessness and headache, have been reported after exposure to 0.5 ppm ozone for up to 6 hours (67). Increased bronchial reactivity has been observed after exposures to similar ozone concentrations for only 2 hours (112). No change in lung function (FEV_1) was seen in subjects exposed for 2 hours (resting) to ozone concentrations below 0.4 ppm (89). Nor was any effect observed on lung function or airway sensitivity to cold air when 24 healthy men were exposed to ozone concentrations of 0.08 – 0.16 ppm (intermittent exercise). In this study exposure to 0.16 ppm ozone was irritating to respiratory passages (82). In studies by Adams (2) the experiments were designed with 6.6 hours of exposure to 0.04, 0.06, 0.08, or 0.12 ppm. No significant effects on lung function or symptoms were observed at the two lower concentrations. A significant reduction in FEV_1 and more pronounced symptoms of irritation (combined throat irritation, cough, breathlessness and pain while taking a deep breath) were seen at 0.08 ppm, and significant effects were also reported at 0.12 ppm. In two other studies, exposure to 0.08 ppm ozone for a total of 6.6 hours (5 hours of exercise) resulted in significant effects on both lung function and airway sensitivity (54, 88), and one of these studies (88) also reported respiratory symptoms. Dose-related responses have also been demonstrated for several lung function variables and symptoms when ozone exposure (0.15 – 0.25 ppm) was combined with intermittent but very strenuous exercise (75). Effects on lung function thus increase with ozone concentration, workload and exposure time.

The effects of ozone seem to diminish with advancing age (89). In one study, 146 men and 94 women were exposed to 0.42 ppm ozone for 1.5 hours. FEV_1 dropped by 16% in young men and women (below 35 years of age); by 12% for middle-aged men (over 35); and by 6% for middle-aged women (48).

Frampton *et al.* (41) found that after exposure to 0.22 ppm for 4 hours (exercising), healthy smokers reported fewer symptoms and showed less effect on airway sensitivity than healthy subjects who had never smoked. FEV_1 dropped by more than 15% for 29% in the latter group, but only for 12% of the smokers. It was also found that ozone sensitivity decreased (>15% reduction of FEV_1) with increase in the number of pack years (a measure of cumulative tobacco consumption: one pack year = 1 pack per day for one year or 2 packs per day for half a year). This may be a result of selection, i.e. smokers may be less sensitive people (41). This explanation, however, is not supported by an earlier study (38) in which 10 smokers were exposed (0.4 ppm for 2 hours) before and after a 6-month smoking-free period. Before the no-smoking period the ozone exposure had

no discernible effect, but afterward there was a drop in forced expiratory flow (FEF_{25-75}).

Brief, repetitive exposure to 0.2 ppm ozone over a period of four or five days results in adaptation: acute symptoms and effects on lung function and airway sensitivity become less severe. There are also declines in some inflammatory markers, though there is still inflammation (64). The tolerance can last for 7 to 20 days, although individuals are very different in this respect (19, 35, 55). This adaptation has been studied mostly in animal experiments (32, 59, 68) (see below under "Animal data").

Higher levels of inflammatory cells, mostly neutrophilic granulocytes, as well as various inflammatory markers (e.g. prostaglandin E_2, interleukin 6 and 8) have been measured in both the central (biopsy) and peripheral airways (BAL, broncho-alveolar lavage) following ozone exposure. These inflammatory changes could be identified as early as one hour after 1 – 2 hours of exposure (strenuous exercise) to 0.3 – 0.4 ppm ozone (73), and increase in neutrophilic granulocytes could be seen after 1 hour of exposure to 0.15 ppm (111). Similar effects on BAL, with increased proportions of neutrophilic leukocytes and interleukin 6, were demonstrated in another study with even lower ozone concentrations: 0.08 ppm for 6.6 hours (strenuous exercise) (34). On the other hand, Blomberg *et al.* could not find neutrophilia in either airways or airway muscles (biopsy) of subjects exposed to 0.2 ppm ozone for 2 hours (light exercise) (15). The authors suggest that this difference from other exposure studies may be due to the relatively low ozone dose. No significant difference in exhaled nitric oxide (NO), a marker of oxidative stress and inflammation, could be shown when eleven healthy subjects were exposed to 0.2 ppm ozone for 2 hours and compared to inhalation of fresh air (100). Nor has any clear correlation been established between changes in lung function and increase in early inflammatory markers (9).

Genetic factors influencing sensitivity to ozone exposure have also been studied. In one study (30), 22 volunteers were exposed to 0.1 ppm for 2 hours (intermittent exercise) and biomarkers for inflammation and oxidative stress were analyzed in blood and condensate from exhaled air. Large individual differences in levels of biomarkers were observed both before and after the exposure. The greatest effect of the ozone exposure was seen among the subjects (n = 8) who had the "wild type" genotype combination (187 Pro) for NAD(P)H:quinone oxidoreductase (NQO1wt), and lacked glutathione transferase M1 (GSTM1null). These results support an earlier study by the same group (14), where effects on lung function and serum levels of lung-specific Clara cell protein (CC16, an early marker of damage to pulmonary epithelium) were measured before and after 2 hours of cycling in ambient ozone levels of 0.032 to 0.1 ppm. With cycling where the ozone level exceeded 0.08 ppm, decline in lung function and increase in serum levels of CC16 were seen in the group of subjects with the genotype NQO1wt and GSTM1null, whereas only the CC16 increase was seen in the groups with other genotypes. No relation was seen between CC16 in serum (indicating epithelial damage) and reduced lung function after exposure to 0.2 ppm ozone for 2 hours

(16). The levels of 8-OHdG (a biomarker for interaction between ROS and DNA) also rose in the "sensitive" genotype group (30). That GSTM1 can be significant was also suggested in a study of asthmatic children in Mexico City, where the effect of ozone on forced expiratory flow (FEF_{25-75}) was greater (-2.9% per 0.05 ppm ozone) in children lacking GSTM1 than in children having this gene (-0.6% per 0.05 ppm) (107). The genotype (-308 G/A) for TNF-α (Tumor Necrosis Factor α) has recently been shown to affect the reduction of FEV_1 after controlled exposure to ozone (0.2 – 0.4 ppm, 2 – 4 hours, intermittent exercise, asthmatics and healthy nonsmokers) (132).

Ozone has been shown to enhance asthmatic reactions in previously sensitized persons. Some studies show that asthmatics react with greater airway obstruction than healthy subjects. Two hours of intermittent exposure to 0.4 ppm ozone resulted in greater reduction of FEV_1 and forced expiratory flow in a group of asthmatics than in healthy controls (74), although there were no differences in effects on lung volumes and bronchial reactivity. Another study revealed a difference between asthmatics and healthy subjects in inflammatory response (sputum), but not in lung function. The effects were seen after 1 hour of exposure to 0.25 ppm ozone (rest or light exercise), but not at 0.125 ppm (52). After a few hours of exposure to 0.16 ppm, wheezing was reported in 9 of 17 asthmatics but in none of the 13 non-asthmatics (53). Alveolar macrophages, but not neutrophilic granulocytes, increased in the lower respiratory passages of asthmatics after exposure to 0.2 ppm for 4 hours/day on 4 consecutive days (7). For people with allergic asthma, ozone exposure enhances the increase of eosinophilic granulocytes if the exposure is preceded by an allergen exposure, indicating a synergistic effect (124). Data in this study also indicate that the profile of the inflammatory effect of ozone exposure can change (eosinophilic instead of neutrophilic) if the exposed person has bronchial inflammation.

A survey article published in 1981 concluded that experimental exposures have provided no definite evidence of a synergistic effect between ozone, sulfur dioxide and nitrogen dioxide (40). However, airway obstruction was measured in asthmatics in a study in which 13 asthmatics aged 12 to 18 were exposed to 0.12 ppm ozone in combination with sulfur dioxide (70). In this study, exposure to 0.12 ppm ozone for 45 minutes followed by exposure to 0.1 ppm sulfur dioxide resulted in a significant decrease of FEV_1, but no effects were observed after exposure to either substance alone. Eight atopics were exposed for 3 hours in an exposure chamber to 0.3 ppm ozone or 75 μ/m^3 office dust or both. The single exposures had little or no effect, but significant effects were observed after the combined exposure (93).

Studies of exposed workers

Welders are exposed to a mixture of metal particles as well as to gases such as ozone, nitric oxide (NO), nitrogen dioxide (NO_2) and carbon monoxide (CO). The highest ozone concentrations measured around welding were 0.1 – 0.6 ppm, in connection with gas-shielded welding in aluminum and stainless steel (11, 72, 113). There are studies from the 1950s reporting much higher concentrations,

between 1 and 5.5 ppm (45). In the light of present experience, these figures seem unreasonably high.

Symptoms of chronic bronchitis (mucous cough, chest tightness) were reported in more welders, especially smokers, than controls (17). The complex exposure of welders makes it difficult to identify a causal relationship between symptoms and (in this case) ozone. Sjögren and Ulfvarson, however, were able to show that, with welding in aluminum, respiratory symptoms increased significantly with ozone concentration but not with particle content (113).

Pulp bleachery workers are normally exposed to low ozone concentrations (below 0.04 ppm), but brief, high exposures occur several times per month due to accidents and gas leaks (98). Workers exposed repeatedly to such exposure peaks showed indications of inflammation in respiratory passages. Exhaled NO for these workers was 90.0 nL/min, compared to 58.8 nL/min for workers without such exposures (99). A study of 228 workers in three pulp bleacheries reports a higher occurrence of asthma symptoms, including breathlessness and wheezing (98), as well as elevated occurrence of asthma (97). In a follow-up study it was found that chronic airway obstruction was more common among the workers who had reported in the first study that they had previously been exposed to exposure peaks (16.1%) than among the other workers (3.7%) (91). It should be mentioned that several of the subjects in these studies had previously been exposed to chlorine dioxide (ClO_2) or sulfur dioxide (SO_2) (49). These substances are risk factors for chronic bronchitis but not for asthma.

About 8% of airplane crew members reported moderate or severe respiratory symptoms in connection with high-altitude flights, but there were no effects on lung function (about 2 weeks after the flights) (118). The reported ozone concentration was 0.4 ppm (2-hour average). In a similar study, flight attendants in high-altitude flights reported symptoms three or four times more often than personnel in low-altitude flights (106). Declines in FEV_1 were measured in 6 persons who were exposed to 0.5 ppm ozone 3 hours/day, 6 days/week during 12 weeks of flying. However, if the ozone level was below 0.2 ppm there was no visible effect on lung function (45). Low humidity (1 – 27%, average 6%) and wide temperature fluctuation (17.4 – 26.8°C, average 22.2°C) also affect the air quality in the cabin and the occurrence of symptoms (80). In another study, flight attendants reported more severe symptoms than either pilots or a control group of office workers. Atopy and passive smoking were major factors (81). Although smoking is now prohibited on most flights, it is still possible that ozone is not the only environmental factor that may cause symptoms in both passengers and crew.

Ozone-generating equipment has been used in cleaning to remove tobacco odor and other undesirable smells from hotel rooms, etc. Cleaning personnel working in rooms where an ozone generator was running, or who entered the room soon afterward (how long afterward not reported), have reported irritation of eyes and/or throat. In an experimental situation, ozone concentrations of 2 ppm or higher were measured in a room during ozone treatment, and 0.2 and 0.1 ppm 10 and 15 minutes afterward, respectively (117).

Studies of ambient ozone

Demographic studies have been used to study exposure to air pollutants, usually including measurements of ozone (O_3), nitrogen dioxide (NO_2) and particles ($PM_{2.5}$ or PM_{10}). In studies of short-term exposures to ozone the variables studied are usually symptoms and lung function, and these studies have usually been made in places where exposure levels are high (summer). Longitudinal studies concentrate on effects of long-term exposure, which appear as chronic diseases (21, 22).

Three studies have been made on whether lung development in young people is affected by the ozone content of outdoor air, and they arrived at different results. Tager *et al.* (116) found that the average content of ozone had affected FEF_{25-75} and FEF_{75} (which reflect resistance in peripheral airways) in persons who grew up in California, whereas no such effect was seen in another study of adolescents (10 to 18 years old) (44). The Tager study found the effect primarily in persons with small peripheral airways (FEF_{25-75}/FVC = airway resistance related to lung volume). An Austrian study found that lung growth in school children is affected by summertime levels of ozone (42).

Differences between healthy persons and persons with respiratory diseases such as asthma have also been the subject of special study. Several studies indicate that some new cases of asthma, and especially more pronounced symptoms and more frequent attacks (and thus more medication and more hospital visits), can be related to ozone levels (119). One study showed an elevated risk of developing asthma for men who lived and worked in areas with high ozone concentrations (87). Children with asthma or respiratory symptoms do not show a greater absolute decline in lung function than healthy children, but can nevertheless show greater sensitivity because of their lower base values. McConnell *et al.* showed that children who played outdoor sports ran a greater risk of developing asthma, and that the total time they spent outdoors also had some effect (86).

Mortality due to heart and lung disease resulting from ozone exposure is another variable that has been examined in epidemiological studies (for example, *Epidemiology* had a theme issue, Volume 16, 2005; References 13 and 58). A European study (APHEA-1, Air Pollution and Health, a European Approach) revealed that the daily mortality rate in 6 cities rose by 2.9% (95% confidence interval: 1.0 - 4.9) with each 0.025 ppm increase in the ozone level, measured as the maximum 1-hour ozone concentration (121). In a later, expanded study covering 23 cities (APHEA-2) some effect of ozone on mortality could also be shown, but only during the warmer part of the year (46). A large American study (NMMAPS, the National Mortality, Morbidity and Air Pollution Studies) also showed a weak correlation between ozone exposure and mortality during summer (108). A 0.01 ppm increase in ozone increased mortality by 0.41%. For air quality standards for ambient ozone in the USA, see further under “Occurrence, use.”

Ozone is one of several different substances in environmental air pollution and has often been used as an indicator for chemical oxidants, but WHO (128) has published the opinion that ambient ozone itself has an effect. In the most recent

WHO air quality guidelines the maximum daily ozone level (8-hour average) has been reduced from 0.06 ppm to 0.05 ppm (128). Studies of the effects of ozone in the general environment differ from occupational exposure studies in that the general population contains a higher proportion of sensitive persons than the working population.

There are also some short-term studies of ozone exposure in various environments. In Holland, a group of 25 amateur cyclists (average age 25, range 18 – 37) were examined several times before and after both training (heart rate average 161 beats/minute, average ventilation 70 l/minute) and racing (heart rate 176 beats/minute, average ventilation 90 l/minute). The training sessions and the races were on average 75 minutes long, and the average ozone level was 0.0435 ppm (range 0.013 – 0.0975 ppm). There was a significant correlation between decline in lung function (FVC, FEV_1, PEF) and ozone level, even when ozone levels above 0.06 ppm were excluded from the calculations. When levels of 0.04 ppm or above were excluded, however, no effect was noted. Effects on lung function were more pronounced early in the summer than later on. Reported respiratory symptoms (breathlessness, difficulty breathing, wheezing) increased in frequency (only breathlessness significant) with increasing ozone levels up to 0.1 ppm (20). In another study, Spektor *et al.* documented lung function declines at these ozone levels in children staying 4 weeks at a summer camp in New Jersey (114). They also demonstrated declines in lung function in 30 healthy, nonsmoking adults who exercised outdoors for a few hours daily during a summer (ozone levels below 0.08 ppm) (115). The duration and intensity of the exercise sessions varied quite a bit (ventilation 20 – 153 l/minute, length of exercise session 15 – 55 minutes). Lung function in 58 seasonal workers (average age 44, range 10 – 69) picking berries (regarded as medium-heavy work, average ventilation 30 l/minute) was monitored for 2 summer months in Canada (18). The daily maximum environmental exposure to ozone was on average (1-hour averages) 0.04 ppm (range 0.013 – 0.084 ppm). There was a significant correlation between ozone level and decline in lung function (FEV_1 and FVC) during the day. The effect lasted until the following morning. The lung function decline during the day was still significant when only days with ozone levels of 0.04 ppm or lower were used in the calculation (18). Children at a summer camp showed indications of inflammation in respiratory passages (significantly elevated NO values in the evenings, compared with exhaled NO in the mornings) when the ozone level was 0.07 ppm or higher for 8 hours/day. The changes in NO were not correlated to lung function (95).

Animal data

The mechanisms behind ozone-induced inflammation and hyperreactivity in airways and the underlying causes of tissue damage (hyperplasia and hypertrophy) in the lungs have been studied in several animal experiments (27). Early changes resulting from ozone exposure vary from species to species and from strain to strain.

Characteristic of the acute effect is that it is often delayed after exposure to 0.5 ppm or less, while concentrations above 0.8 ppm yield immediate pro-inflammatory changes. A cachexia-like condition appeared in mice after 3 nights of (8-hour) exposure to 1 ppm ozone. The mice lost 14% of their original weight, and there was down-regulation in function of several different genes including interferon-dependent genes in the liver (77). In rats, a background exposure of 0.06 ppm ozone combined with higher exposure for 9 hours/day 5 days/week, produced acute tissue reactions with epithelial inflammation, interstitial edema and cell hypertrophy, as well as invasion of macrophages (25). (Daily exposure was designed to simulate urban exposure: 15 hours at 0.06 ppm, gradually increasing to 0.25 ppm over 4 1/2 hours, thereafter decreasing over 4 1/2 hours to 0.06 ppm.)

Chronic exposure to the same low level of ozone caused epithelial inflammation and interstitial fibrosis in the proximal alveolar region, as well as invasion of bronchial epithelial cells. No fibrotic changes could be observed when mice were exposed to 0.2 or 0.5 ppm ozone (78). Effects on lung morphology and function were seen in macaques after exposure to 0.15 ppm or 0.3 ppm ozone 8 hours/day for 6 or 90 days. Hyperplasia and hypertrophy of cuboidal cells in the bronchioles were observed after only 6 days of exposure at the lower level (47).

Several factors have been found to be necessary for the invasion of neutrophilic granulocytes and thus bronchial hyperreactivity: induction of the chemokine receptor CXCR2, which is expressed in neutrophils, monocytes and T cells (61), as well as the pro-inflammatory cytokine TNF-α (28). Activation of the complement system, which induces neutrophilic inflammation and hyperreactivity in air passages, was also seen in mice after 3 hours of exposure to 2 ppm ozone (102). Eosinophilic granulocytes were found to have double roles after acute ozone exposure of guinea pigs to 2 ppm ozone for 4 hours. Eosonophils were active in the appearance of hyperreactivity in respiratory passages at an early stage and later with repair of the damaged parasympathetic nerves (133). An increase of alveolar macrophages was demonstrated in rabbits 7 days after a single 2-hour exposure to 0.1 ppm ozone (36).

Nitric oxide (NO) affects inflammation and causes vasodilation and bronchodilation. Different isoforms of NO-synthase (NOS) were measured in lung tissue of mice after exposure, and there was found to be an increase of eNOS (endothelial) and nNOS (neuronal), which would probably increase sensitivity in air passages after exposure to ozone (60).

Ozone tolerance, with gradual decline of markers for inflammation and oxidative stress, was shown in an exposure experiment in which 6 calves were exposed to 0.75 ppm ozone 12 hours/day for 7 days (68). Adaptation of methacholine sensitivity in respiratory passages of mice exposed to 2 ppm ozone for 4, 8 or 12 weeks has also been documented (59).

Genetic factors have also been studied in animal experiments, to identify possible groups with higher risk of negative effects of ozone (132). Clear genetic causative correlations have been found to reactions to ozone in several animal

species, and some of the genes involved (e.g. TNF-α) have also been identified (110). Toll-like receptor 4 (Tlr-4, also involved in hereditary immunity and endotoxin sensitivity) appeared as a strong genetic candidate for the variation in ozone-induced lung hyperpermeability in a study in which mice were exposed to 0.3 ppm ozone for 72 hours (69).

It has also been shown in animal experiments that respiratory allergies are a risk factor with repeated ozone exposure. The antigen-presenting activity, measured as expression of antigen-presenting molecules on cell surfaces (Ia, B7.1, B7.2 and CD11b/c), increased in allergen-induced animals (with ovalbumin, OVA) after exposure to different doses of ozone (0.3 – 1 ppm). It has been proposed that this increased activity enhances allergic symptoms (71). Allergy-like symptoms involving the nose, caused by infiltration of eosinophilic granulocytes, also showed a dose-dependent (0.1 – 0.6 ppm ozone) increase in allergen-induced (OVA) guinea pigs (57). Hyperplasia in alveolar epithelium with increased airway resistance and reduced dynamic compliance was also demonstrated in allergen-induced (OVA) mice after 5 weeks of ozone exposure (1 ppm) (43). On the other hand, ozone (0.3 ppm) had no effect on development of emphysema in mice in which emphysema had been induced by cigarette smoke (84).

Interacting mechanisms have also been studied in animal experiments. Endotoxin and ozone exposure together enhance and prolong the inflammatory process, with elevated levels of chemokines and cytokines and more severe edema development in the lungs (62, 125). Rats exposed to Cr(VI) levels similar to occupational exposure (360 μg Cr/m^3) in combination with 0.3 ppm ozone showed changes in mechanisms behind lung clearance of Cr particles (particle uptake and processing by alveolar macrophages). This ozone-related change in clearance may well constitute an increased risk to health (carcinogen) for welders exposed to insoluble and sparingly soluble chromium compounds (29).

It was shown in rats that damage occurs further down in the respiratory passages when exposure occurs during exercise (walking) (85). It was also demonstrated in this study that ozone and formaldehyde have an additive effect.

Other effects

In a study of photocopying in poorly ventilated rooms (134) a correlation was found between higher levels of ozone and lower levels of antioxidants in the body. Eighty nonsmokers who made copies were compared with 80 controls in this study, and ozone levels ranged from 0.05 to 0.2 ppm. Exposure measures in this study, however, are inadequate for determining a dose-response relationship.

Effects on the circulatory system, notably on heart rate and blood pressure, have been shown in rats experimentally exposed for 3 hours to 1 ppm ozone, whereas animals exposed to air were unaffected. ECG effects were also observed (122). These researchers also found that the number of bradycardia episodes increased with ozone concentration, and that there was a simultaneous effect on brain activity (6). Indications of extra- and intracellular edema were seen in the hearts

of rats after exposure to 0.7 ppm ozone for 5 days. These results indicate that exposure to this much ozone probably affects both heart and brain (105).

Sleep disturbances have been observed in animals exposed to ozone. They may be due to physiological and biochemical changes (e.g. catecholamines, serotonin, acetylcholine) in the central nervous system. In one study, disturbance of the diurnal sleep cycle was observed in rats after 24 hours of exposure to 0.5 ppm ozone. Disturbances in heart rhythm and acetylcholine excretion were also observed (3).

Oxidative stress in the skin as an effect of ozone exposure was studied by Valacchi *et al.* Oxidation of lipids and proteins in the skin was observed in a study in which an area of mouse skin was exposed to 8 ppm for 2 hours (127). In a later study by this group, mice were exposed to 0.8 ppm ozone 6 hours/day on 6 consecutive days. The exposure induced pro-inflammatory mediators and protective proteins in both lungs and skin (126).

Mutagenicity, carcinogenicity, effects on reproduction

Ozone is mutagenic to the bacteria *E coli* (76) and *Salmonella* (129) as well as the yeast *Saccharomyces cerevisiae* (37). DNA damage caused by ozone is also mutagenic in human cells (293-KMT11 cell line). *In vitro* exposure of plasmid DNA to 20 ppm ozone for 10, 30 or 60 minutes, followed by transfection of the plasmid to the cells and subsequent DNA replication, resulted in mutations in the human cell line. The mutations were of the same type as those caused by reactive oxygen intermediates (63). Genotoxic effects have been demonstrated in cell cultures at ozone levels of 0.15 – 1 ppm (22).

Adenomas were induced in the lungs of A/J mice, which are particularly susceptible to developing lung adenomas, but not Swiss-Webster mice, which are more resistant. A significant increase was seen after exposure to 0.8 ppm, 8 hours/night, 7 nights/week for 18 weeks, but no increase was observed after exposure to 0.4 ppm on the same schedule (79). In another study, female A/J mice were exposed for a total of 9 months to 0.12, 0.5, or 1 ppm ozone and the ozone exposure had no observable carcinogenic effect (130). In an NTP study, $B6C3F_1$ mice were exposed to 0.06, 0.25 or 1 ppm ozone 6 hours/day, 5 days/week for 105 weeks (2 years) or 130 weeks (lifetime; only the two higher exposure groups). At the highest exposure a significant increase of alveolar/bronchiolar adenoma and carcinoma was seen in the females, and a not significant increase in the males, when compared with unexposed controls (51, 96). No increase in tumor incidence was seen in F344/N rats after 105 or 125 weeks at these exposures. Nor was there an increase of NNK (4-[N-methyl-N-nitrosamino]-1-[3-pyridyl]-1- butanone)-induced lung tumors in male rats exposed to 0.5 ppm ozone (96). According to the NTP's own assessment, the study provides "equivocal evidence" of carcinogenic activity for male and "some evidence" for female $B6CF_1$ mice but "no evidence" for the F344/N rats (96). Tumors have not been found in either rats or hamsters. No tumor formation in either peripheral lung tissue or nasal cavities was seen in hamsters exposed for 6 months to 0.8 ppm ozone (131). In another study in which

rats were exposed to 0.05 ppm ozone 10 hours/day for 13 months, no adenomas or carcinomas were observed (56). However, if the rats were treated with N-bis(2-hydroxypropyl) nitrosamine (BHPN) before exposure to ozone and nitrogen dioxide, there was a significant increase in tumor formation. Since tumors occurred in only one species, it is difficult to assess these studies in terms of risk to humans (22).

No significant increase in the number of chromosome aberrations could be seen in 30 volunteers exposed to 0.4 ppm ozone for 4 hours (90). In subjects exposed to 0.1 ppm ozone for 2 hours (intermittent exercise) there was an increase of 8-hydroxy-2´-deoxyguanosine (a biomarker for interaction between ROS and DNA) (30).

One population study showed a significant positive correlation between ozone level and the level of DNA adducts in blood of adults in Florence (101), and in another study indication of genetic damage in the form of higher occurrence of micronuclei was seen in cells from buccal epithelia of the students who spent the summer in a city with high ozone levels (on average 50 days above the exposure limit of 0.09 ppm) when they were compared with students staying in a city with lower ozone levels. Further, there was an increase of micronuclei in cells from buccal epithelia of 15 students exposed to 0.2 ppm ozone for 4 hours (26). More single-strand breaks in DNA from nasal epithelium in both children and adults exposed to air pollutants with high levels of ozone in Mexico City (average 0.25 ppm), compared with a control group, may be evidence that ozone exposure leads to DNA fragmentation (23).

Relationships between mortality/lung cancer incidence and exposure to airborne particles (PM_{10}), sulfur dioxide and ozone in ambient air were studied in a cohort of 6338 nonsmokers (Seventh Day Adventists) in California (1, 12). Among the men both lung cancer incidence and mortality increased with increasing exposure to PM_{10}, sulfur dioxide and ozone. Among the women the correlation to ozone was weak. The authors point out that interpretation is difficult since there was strong co-variation in the levels of PM_{10} and ozone, but an analysis model that included both pollutants indicated that ozone probably has its own carcinogenic effect. In the large American Cancer Society study, covering about 500,000 adults in the U.S., there was no observed correlation between ozone level and mortality from lung cancer (103).

Ozone has not been classified for carcinogenicity by either the IARC (International Agency for Research on Cancer) or the EU.

When mice were exposed during gestation to 0.1 – 0.2 ppm, 7 hours/day, 5 days/week, neonatal mortality was higher (45). Abnormal uterine contractions were observed in rats on days 5 and 10 of gestation after exposure to 3 ppm ozone (24).

Dose-effect/dose-response relationships

Correlations between effects and human exposure to various air concentrations of ozone are summarized in Table 1. Table 2 summarizes effects observed in experimental animals exposed to low levels of ozone.

Symptoms (coughing and difficulty inhaling), increased reactivity and acute reduction in lung function (7 to 8%) could be correlated to ozone exposures of 0.08 ppm in controlled experiments (see Table 1). On the other hand, in a study in which ozone levels were 0.06 ppm (light exercise) the exposure had no observed effect. Ozone sensitivity varies considerably from person to person. At higher concentrations (0.16 ppm), symptoms are more pronounced (wheezing) and persons with asthma and/or chronic obstructive pulmonary disease (COPD) experience more attacks leading to more emergency hospital visits.

There are some areas of agreement between studies of occupationally exposed persons and laboratory studies of people and animals. They show that ozone exposures yield inflammatory changes in respiratory epithelium. Increased numbers of neutrophilic granulocytes have been found in both rabbits exposed to 0.1 ppm and human studies with exposures as low as 0.08 ppm. The increased antigen-presenting activity measured in allergen-induced animals shows that respiratory allergy is a risk factor for exacerbation of symptoms for people with allergies and repeated exposure to ozone.

Whether or not ozone is carcinogenic to humans can not be determined. Ozone is mutagenic in *in vitro* tests, and DNA damage has been observed in people exposed to an ozone concentration of 0.1 ppm. Tumor occurrence has been studied in laboratory animals, but elevated tumor frequency was seen only in one species (mice). A statistical correlation has been found between ozone concentration and increased cancer morbidity and mortality in men, but other exposures may have been involved.

For workers with jobs involving exposure to low ozone concentrations, effects are seen primarily as increase of various respiratory symptoms. Chronic airway obstruction has been observed in bleachery workers who have been exposed to brief ozone peaks from gas leaks, etc. Complex and co-varying exposures are common, as with welding, and make it difficult to establish a dose-effect or dose-response relationship for ozone.

Correlations between ozone levels in outdoor air and mortality, morbidity and respiratory symptoms have been found in epidemiological studies. There are also studies suggesting that ozone levels can affect lung development. In some of these studies, effects of ozone have been observed at lower levels (below 0.06 ppm) than in experimental studies. An explanation for this difference may be that epidemiological studies include individuals who are more sensitive (children, old people, sick people). Short-term studies with ambient exposure also have shown effects of ozone on both children and adults. In a study of amateur cyclists, declines in lung function were seen during very heavy exercise (bicycle racing/training) at an exposure level of ≤0.06 ppm ozone, and at ≤0.04 ppm

in a study of seasonal workers (berry pickers) with medium-heavy work. The low effect levels compared to exposure chamber studies might be explained by the higher workload in these short-term studies. It has also been proposed that other environmental factors may potentiate the effects of the ozone exposure (115). Transferring these observations from ambient exposure to occupational exposure is made even more difficult by the fact that ambient ozone is measured as a marker for a pollutant situation that may be considerably different from a work environment. Ozone is one of numerous pollutants, and they may co-vary with the ozone.

Conclusions

The critical effect of exposure to ozone is judged to be respiratory symptoms and decline in lung function. In laboratory studies, these effects have been documented at ozone levels as low as 0.08 ppm for 6 hours of light exercise. In short-term studies with ambient exposure, where ozone measurement is used as an indicator for general pollutant levels, effects on lung function have been observed at lower levels.

Table 1. Effects observed on volunteers exposed to low levels of ozone in exposure chambers and in short-term studies of ozone exposure in the ambient environment.

Exposure			Subjects	Effects	Ref.
ppm	Hours	Workload[1]			
0.4	1– 2	Resting	13	No effect on FEV_1.	89
0.4	2	Resting	30 men	No increase in number of chromosome aberrations.	90
0.4	2	Medium, 1 hour	7 men 3 women	Increased bronchial reactivity (methacholine).	112
0.22	4	Heavy	56 nonsmokers, 34 smokers	Smokers had fewer symptoms and less reduction of FEV_1 than those who had never smoked.	41
0.16	7.6	Light	17 asthmatics 13 healthy	Asthma symptoms (wheezing) in 9 of 17 asthmatics.	53
0.16	2	Very heavy, 1 hour	24 men	Irritation in respiratory passages.	82
0.15	1 (3 days)	Resting	5	Increased numbers of neutrophilic granulocytes in lower respiratory passages.	111
0.15	2	Very heavy, 0.9 hour	20 men	Concentration-related effect on respiratory flow, conductance and total lung capacity (TLC); respiratory symptoms.	75

Table 1. Cont.

Exposure			Subjects	Effects	Ref.
ppm	Hours	Workload[1]			
0.12	0.75	Resting	Asthmatics: 8 men 5 women	Greater reduction of FEV_1 and V_{max} with exposure to O_3 + SO_2 than with O_3 only.	70
0.1	2	Light intermittent	12 men 10 women	DNA damage. Changes in biomarkers for inflammation and oxidative stress.	30
0.08	6.6	Heavy	38 men	Increased bronchial sensitivity and symptoms. FEV_1 reduced 8.4%.	88
0.08	6.6	Medium	22 men	Decline in lung function (7.1%). Increased bronchial reactivity (methacholine).	54
0.08	2	Very heavy 1 hour	24 men	No significant decline in lung function or increase in bronchial reactivity (cold air). Slightly irritating.	82
0.08	6.6	Heavy	18 men	Increase of neutrophilic granulocytes, PGE_2 , LDH and IL-6 in BAL.	34
≤0.08[2]	29 min (15-55)	Light to very heavy	20 men 10 women	Declines in lung function.	115
0.08	6.6	Light	15 men 15 women	Decline in lung function, respiratory symptoms.	2
0.06	6.6	Light	15 men 15 women	No symptoms, no effect on lung function.	2
≤0.06[2]	75 min (10-145)	Very heavy (cycling)	29 men	Decline in lung function.	20
≤0.06[2]	4 weeks	Summer camp	53 boys 38 girls	Decline in PEF values.	114
≤0.04[2]	11 h/day 2 months	Medium	26 men 32 women	Decline in lung function.	18
0.02			10	9 of 10 notice the smell.	50

[1] Workload classified in accordance with AFS 2005:17 (5).
[2] Ambient air exposure.

Table 2. Effects observed in laboratory animals exposed to low concentrations of ozone.

Exposure		Species	Effect	Ref.
ppm	Time			
1.0	5 days/wk 5 weeks	Mouse (allergen-induced)	Hyperplasia in alveolar epithelium. Increased airway resistance.	43
1.0	3 hours	Rat	Effects on heart rate and blood pressure.	122
1.0	6 h/day 5 days/wk 130 weeks	Mouse	Significant increase in pulmonary adenomas and carcinomas.	51
1.0	8 h/night 3 nights	Mouse	Weight loss.	77
1.0	6 h/day 5 days/wk 9 months	Mouse (A/J females)	No carcinogenic effect.	130
0.8	6 h/day 6 days	Mouse	Induction of pro-inflammatory mediators in lungs and skin.	126
0.8	8 h/night 18 weeks	Mouse (A/J)	Increase of adenomas in lungs.	79
0.8	6 months	Hamster	No adenomas or carcinomas.	131
0.7	5 days	Rat	Morphological changes in heart.	105
0.5	3 x 1 hr/week 2 weeks	Mouse	No fibrotic changes.	78
0.5	24 hours	Rat	Disturbance of diurnal sleep cycle.	3
0.3	72 hours	Mouse	Lung hyperpermeability.	69
0.3	7 h/day 3 days x 3 (2-week intervals)	Rat (allergen-induced)	Increased antigen-presenting activity.	71
0.3	8 h/night 5 n/week up to 32 weeks	Mouse	No effect on development of emphysema.	84
0.06 + 0.25 (max)*	1 week or 78 weeks 5 days/wk	Rat	Acute tissue reaction (1 week). Fibrosis (78 weeks).	25
0.2	7 hrs/day 5 d/week entire gestation	Mouse	Increased neonatal mortality.	45
0.15	8 h/day 6 days	Monkey	Hyperplasia and hypertrophy in terminal bronchioles.	47
0.1-0.6	5 weeks	Guinea pig (allergen-induced)	Dose-dependent nasal symptoms and increase in eosinophils.	57
0.1	2 hrs	Rabbit	Increase in alveolar macrophages.	36
0.05	10 h/day 13 months	Rat	No adenomas or carcinomas.	56

*In one day of exposure the animals were exposed to 0.06 ppm for 15 hours. Over the next 9 hours the concentration was gradually increased to 0.25 ppm and then gradually decreased back to 0.06 ppm.

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Consensus Report for Nitric Oxide

June 13, 2007

This document is based on a WHO review published in 1997 (19), and on subsequently published literature. A literature search was made in December of 2005, and has been supplemented with articles published since that time. The Criteria Group published its previous Consensus Report for nitric oxide in 1986 (23).

Chemical and physical data. Occurrence

CAS No.:	10102-43-9
Synonyms:	nitrogen monoxide, nitrogen oxide
Molecular weight:	30.01
Formula:	NO
Melting point:	- 163.6°C
Boiling point:	- 151.8°C
Relative density:	1.04 (air = 1)
Conversion factors:	1 mg/m^3 = 0.83 ppm
	1 ppm = 1.23 mg/m^3

Nitric oxide (NO) at room temperature is a colorless, odorless gas. It is formed during combustion in air (e.g. in internal combustion engines and with welding) by oxidation of the nitrogen in the air. This results mostly (90 – 95%) in NO, with some (5 – 10%) NO_2. The NO is oxidized to NO_2 by the oxygen and ozone in the air. If 1 ppm NO is added to pure air, half of it (0.5 ppm) is transformed to NO_2 in about ten minutes (leaving 0.5 ppm NO) (35). Nitric oxide is also formed by oxidation of the nitrogen in fuels (such as coal and oil), in fertilizers, explosives and dyes, and around production of chemicals containing nitrogen. Nitric oxide can also be formed in fodder silos. Combustion of fossil fuels and biofuels is by far the largest source (19), and nitric oxide is omnipresent in both indoor and outdoor environments.

Tobacco smoke contains nitrogen oxides. In one study the amount of nitric oxide in tobacco smoke is reported to be 98 – 135 mg/m^3 and the amount of nitrogen dioxide 150 – 226 mg/m^3 (8, cited in Reference 37).

Endogenous nitric oxide formation

Nitric oxide is formed naturally in many places in the body, where it acts as a signal substance with numerous functions (6, 7, 31). Its formation and functions are extremely complex and not yet completely understood. Nitric oxide is formed

in the airways and can be used as a marker for inflammation. The amount in exhaled air is dependent on the speed of exhalation. With an exhaled air flow of 50 ml/s it is on the order of 10 ppb (0.01 mg/m^3) for a healthy person, but around 50 ppb (0.05 mg/m^3) for a person with asthma. Smoking affects the amount of exhaled nitric oxide: smokers, both asthmatics and non-asthmatics, exhale less than nonsmokers (22, 26). The content in nasal sinuses can be 0.5 – 1 mg/m^3. Some medicines (e.g. nitroglycerin) exert their effect primarily via formation of nitric oxide. It has been estimated that the EC_{50} (Effective Concentration) for flow regulation by nitric oxide is 1 to 5 nM (11).

Occurrence in work environments

Occupational exposure is mainly to nitric oxide in engine exhaust, but can also occur around welding, fodder storage and glass blowing (23). In mines, propane is sometimes burned to warm the inflowing air. This forms NO and NO_2. The ratio between the two depends mostly on where in the ventilation system the measurements are made, since the NO soon oxidizes to NO_2.

Very few measurements of nitric oxide are reported in the literature. In fodder silos, levels ranging from 3.7 to 775 mg/m^3 were measured just above the surface several days after filling. Levels up to 6.2 mg/m^3 were recorded in a coal-fired power plant. Levels ranging from 0.7 to 9 mg/m^3 have been reported in mines where diesel vehicles are used (37).

Uptake, biotransformation , excretion

In practice, exposure occurs only to NO in gas form. Studies with human subjects, both resting and exercising, have shown that at levels up to 6 mg/m^3 about 90% of inhaled NO is absorbed. In animal studies the percentage of uptake is somewhat lower, especially with higher exposure levels; this has been attributed to reduced ventilation at higher NO exposure. In experiments with dogs it was found that 73% of the NO did not get past the nasopharynx (19). Endogenous NO formation is sometimes measured as increase in blood nitrite (29).

Toxic effects

As noted above, NO is a naturally occurring substance that is found in low concentrations in most of the organs in the body. Older literature contains case reports of severe poisoning due to exposure in silos and tanks for nitrogen gases. These were probably mixtures of different nitrogen oxides, and possibly other gases as well (19). The literature on nitrogen oxide has been increasing rapidly since the discovery that NO is a naturally occurring signal substance which also has therapeutic uses. A search of MEDLINE for “nitric oxide” yielded more than 60,000 entries added in 1996 or later, and a search further limited by “toxicology” yielded over 1500 references.

As a signal molecule, NO can relax smooth muscles and thus increase blood flow, and can counteract cell proliferation by activating the enzyme guanylate

cyclase. NO is formed in endothelial cells; this is catalyzed by enzymes (NO synthase, or NOS), and (with hypoxia) can also be formed in the body from nitrite and nitrate. NOS is regulated (inter alia) by cytokines in inflammation. NO is reactive and can combine with superoxide to form peroxynitrite, which can initiate lipid peroxidation or damage proteins. The protein damage has been connected to aging. The reaction with superoxide, at least at low NO concentrations, can also counteract oxidative stress. Asbestos can catalyze NO oxidation to nitrite, counteract the signal action of NO, and accelerate protein damage (34).

Inhalation of NO has been used in treating lung disease, for the purpose of dilating the blood vessels in the lungs, usually in concentrations of 5 to 40 ppm (31, 36).

NO can have several, sometimes opposing, modes of action, such as both pro- and antioxidative effects and pro- and anti-inflammatory effects (10, 36). NO can also affect coagulation of blood and cause formation of methemoglobin. When NO is used medically it is often administered along with oxygen, which can accelerate the formation of NO_2. Nitroglycerin produces its therapeutic effect by locally forming NO, which dilates blood vessels. NO helps to regulate several systems, including the nervous system, immune system and cardiovascular system. It can also have complex effects on cell survival, for example both reducing and increasing apoptosis (7).

Effects of short-term exposure on humans

Older literature surveys and risk summaries suggest that 8 hours of exposure to NO at concentrations around 25 ppm may lead to respiratory symptoms including breathlessness and coughing, and that at 100 ppm there is a high risk of lethal damage (7). This information could not be verified in primary references.

In one study, volunteers were exposed for 15 minutes to NO in concentrations ranging from 10 to 39 ppm. Airway resistance increased significantly (about 10%) at exposures above 20 ppm (38, cited in Reference 19). In another study, healthy volunteers were exposed to 1 ppm NO for 2 hours. No significant deviations from normal were seen (20, cited in Reference 19).

NO levels around 5 to 40 ppm have been used in treatment of intensive-care patients with respiratory failure. The treatment may last up to a month, but usually one week is sufficient. At these levels it has been possible to see a positive effect on lung function, reduced inflammation and also lower pressure in pulmonary blood vessels (25, 31, 32). Patients treated with NO often show a rebound effect from the treatment (the blood pressure in the lungs increases dramatically if treatment is stopped abruptly), so the NO is usually reduced slowly (7).

Inflammatory mechanisms are quite important in the development of chronic obstructive pulmonary disease (COPD), and NO is involved in these mechanisms. There are studies reporting elevated levels of NO in exhaled air of persons with COPD, but also studies reporting no increase or even a decrease compared with healthy subjects. Medicines are currently being developed to reduce the formation

of NO in the respiratory passages as a way of limiting the inflammation associated with COPD (5, 24). It has also been suggested that an important function of certain medicines currently in use (cortisone and theophylline) is reducing the formation of NO in the respiratory passages (13). No studies of whether inhaled NO increases the risk of COPD were found.

NO can react with hemoglobin to form nitrosyl hemoglobin, which is rapidly oxidized to methemoglobin. The enzyme methemoglobin reductase reduces the methemoglobin back to Hb (39). The formation of methemoglobin at nitric oxide levels below 100 ppm is usually not clinically relevant for adults (36). For small children the risk can be higher, since they have lower levels of the reducing enzyme.

Medical studies of NO's effects on blood coagulation have yielded contradictory results. In one study no effect was observed after 30 minutes of exposure to 30 ppm NO (1). In another study with healthy men and women, it was found that 20 to 40 minutes of inhalation of 5 ppm inhibited collagen-induced platelet aggregation and also inhibited expression of p-selectin (12). P-selectin mediates the adhesion of leukocytes and platelets to vascular endothelium, and elevated values can be seen e.g. with increased platelet activation (33). At 40 ppm bleeding time was also prolonged.

Animal experiments

The effects of NO have been studied in a very large number of animal experiments, many of them designed to further elucidate the role of NO as a signal substance and to test NO as a medical treatment. The toxicological picture is very complex and can be highly dependent on the NO level and the presence of other factors such as oxygen or bacteria (36).

NO has a bronchodilative effect. In guinea pigs that were first given a bronchoconstrictor, a dose-dependent bronchodilator action was observed from 2.1 ppm up to 300 ppm (9). The effects were almost immediate, and could be demonstrated within 30 seconds.

Mechanically ventilated pigs were exposed to 0, 10, 40 or 80 ppm NO for 24 hours. Those receiving 10 ppm showed no pathological changes when compared with controls. Those receiving 40 or 80 ppm had some edema around alveolar capillaries, and in those receiving 80 ppm there were also effects on pulmonary circulation, with lower arterial pressure (2).

The WHO summary (19) mentions a study indicating that NO increased vulnerability to infections in female mice exposed to 2 ppm NO for 4 weeks. The authors of this study express uncertainty as to the relevance of this observation (3). Methemoglobin formation has been observed to increase in animals exposed to NO levels of 20 ppm or higher (19).

Rats were exposed for 9 weeks to 0.5 ppm NO with exposure highs of 1.5 ppm for 2 x 1 hour per day: they developed changes in lungs resembling the changes associated with emphysema (reduced interstitial volume around alveoli and

reduced number of interstitial cells) when compared with rats exposed to air (controls) and rats exposed to 0.5 ppm nitrogen dioxide (28).

In a subsequent study the same author exposed rats to air alone or to 2 or 6 ppm nitric oxide 22 hours/day for 6 weeks (5 rats per group, NO_2 for exposed rats 0.03 ppm). No interstitial changes were found in the rats exposed to NO, i.e. these results differed from those of the previous experiment. There were, however, a greater number of Type II cells and macrophages in the alveoli of the exposed rats. Exposed animals also had high platelet aggregation in pulmonary capillaries, which surprised the author since he was expecting the opposite effect (27). In his conclusions to the latter study he states that the effects of NO resemble those seen with other oxidants such as nitrogen dioxide and ozone. The mechanism was presumed to be that NO causes inflammatory cells to accumulate in the lungs, and the author suggests that this in combination with pollutants (e.g. particles) may cause lung damage.

Dogs were experimentally exposed to mixtures of NO and NO_2, either 2.05 + 0.27 or 0.31 + 1.2 mg/m^3 (1.7 ppm NO + 0.14 ppm NO_2, or 0.25 ppm NO + 0.63 ppm NO_2) 16 hours/day for 7 years, and then to pure air for 32 to 36 months (18). The experiment also included dogs exposed to various mixtures of engine exhaust. The dogs exposed to the higher dose of NO_2 (0.63 ppm) and the lower dose of NO had significantly larger lungs and indications of destruction of alveolar walls, which the authors interpret as indications of emphysema. There were no significant changes of this nature in the dogs exposed to the higher dose of NO (1.7 ppm). The occurrence of hyperplasia in distal airways (acini) was in general unchanged in the dogs exposed to the higher dose of NO, but increased in the group exposed to the higher dose of NO_2 (but lower NO). In the group exposed to the higher dose of NO_2 there were also indications of cilia loss in major airways, and some metaplasia. There were no significant changes of this nature in the dogs exposed to the higher dose of NO, although there was an elevated occurrence of interalveolar pores. The relevance of this is unclear, however. The authors regard the changes at the higher dose of NO_2 as primarily an effect of the NO_2, but express surprise that the changes were observed at such low doses. This study is difficult to evaluate since it is hard to determine whether the effects are due to the NO_2 or the NO, or to a synergy between the two. Further, there were only a few dogs (6 to 12) per exposure group.

Three of four rabbits exposed to 5 ppm NO for 14 days (nitrogen dioxide level < 0.1 ppm) had indications of edema in alveoli. No such changes were observed in the 4 controls (14).

Rats exposed to 2.0 ppm NO (+ 0.08 ppm NO_2) for up to six weeks showed some changes in alveoli which the authors suspected could be early indications of emphysema, with irregularly occurring alveolar edema (4). The changes were observed only in the animals exposed for five or six weeks. Some similar changes were also seen in controls, but to a lesser extent. The authors express some doubt as to the importance of these changes. No other changes attributed to NO were seen.

After exposure to 15 ppm NO for 10 hours, rats showed prolonged bleeding time and lower platelet aggregation ability (21).

Mice were exposed to 2.4 ppm NO (< 0.04 ppm NO_2) for up to 29 months. At 16 months the exposed animals had a lower survival rate than controls, but at other times there were no differences in either survival rate or body weight. The animals were also examined by various hematological, biochemical, and histological methods at various times, and no changes attributable to NO could be found (30).

Mutagenicity, genotoxicity, carcinogenicity

NO interacts in several ways in the formation of RNA and DNA, and can cause potentially mutagenic structural changes in DNA. Chromosome aberrations after exposure to NO have been demonstrated *in vitro* as well as *in vivo* (36).

Because NO is involved in inflammatory processes, it has been proposed that NO may increase the risk of cancer. Interactions between p53 and NO may be involved in formation of cancer: e.g. p53 inhibits formation of NOS2 (a nitric oxide synthetase) and thus reduces the formation of NO. The connection is extremely complicated, however – for example, tumor incidence in mice without p53 (-/-) but with NOS2 (+/+) was lower than in mice lacking both the p53 (-/-) and NOS2 (-/-) genes (15, 16, 17).

NO has an extremely complex role in the development of cancer. Experimental studies have shown that NO can stimulate formation of new blood vessels, both inhibit and stimulate cell proliferation, and inhibit or stimulate apoptosis, depending on NO concentration and interactions with other substances and molecules (16).

No human studies on cancer risk were found. In an experiment in which mice were exposed to 2.4 ppm NO for up to 29 months, there was no increase in the occurrence of tumors (30).

Effects on reproduction

No information was found.

Dose-effect / dose-response relationships

NO is rapidly oxidized to NO_2 and occupational exposures are therefore usually to both substances. NO is formed in the body, where it is an important signal substance.

There are indications that NO can have positive effects on humans. One such is vasodilation, and NO is used therapeutically in doses of 5 to 40 ppm to reduce pressure in the pulmonary blood vessels of persons with severe lung disease.

Healthy subjects show effects on regulation of coagulation at 5 ppm and prolonged bleeding time at 40 ppm (12). Inhalation of NO results in a dose-dependent formation of methemoglobin. At NO levels below 100 ppm this

methemoglobin formation is regarded as clinically unimportant, although in a few cases higher methemoglobin levels have been reported in very ill patients and infants (36).

Effects on blood coagulation have also been observed in animals. Rats exposed to 15 ppm bled longer (Table 2). Changes in alveoli have been documented in animals exposed for a few weeks to NO levels of 0.5 ppm or above (Table 3). These changes look different in different studies. NO has many toxic mechanisms and can interact with other pollutants and infections, which may be able to explain the differences in results.

Fifteen minutes of exposure to 10 ppm increases airway resistance in healthy subjects. In long-term laboratory exposures, rats show inflammatory changes in alveoli at levels of 0.5 to 2 ppm.

Conclusions

The critical effect of short-term exposure to NO appears to be its effect on blood coagulation and on blood pressure in pulmonary vessels. One study reports a measurable effect on blood coagulation after 20 minutes of exposure to 5 ppm. Increased airway resistance is seen in healthy subjects after 15 minutes of exposure to 10 ppm.

In studies with animals it has been noted that exposure to NO levels of 0.5 to 2 ppm for several weeks results in lung changes at the alveolar level. The types of changes observed are different in different studies, however, which makes interpretation difficult.

Table 1. Effects on human subjects with short-term exposure to nitric oxide.

Exposure (ppm)	Time	Effect	Ref.
1	2 hours	No effect on lung function.	19
5	20 minutes	Inhibited platelet aggregation.	12
10	15 minutes	Increased airway resistance.	19
40	40 minutes	Prolonged bleeding time.	12
80	8 hours	Methemoglobin levels that may cause symptoms in children treated with NO for lung disease.	36

Table 2. Effects on animals with short-term exposure to nitric oxide.

Exposure (ppm)	Time	Species	Effect	Ref.
2.1 to 300	Effect seen after 30 seconds	Guinea pig	Dilation of airways after treatment with methacholine.	9
10	24 hours	Pig	No effect.	2
15	10 hours	Rat	Prolonged bleeding time.	21
40	24 hours	Pig	Slight alveolar edema.	2
80	24 hours	Pig	Slight alveolar edema, reduced arterial pressure in pulmonary arteries.	2

Table 3. Effects on animals exposed to nitric oxide for a few weeks or longer.*

Exposure (ppm)	Time (weeks)	Species	Effect	Ref.
0.5 + 1.5 for 2x1 hr/day	9	Rat	Alveolar changes with reduced interstitial volume.	28
2	6	Rat	Inflammatory changes in alveoli.	27
2	5 - 6	Rat	Alveolar edema and emphysema-like changes.	4
2.4	116 (29 months)	Mouse	No changes.	30
5	4	Rabbit	Alveolar edema.	14
6	6	Rat	Inflammatory changes in alveoli.	27

*In dogs exposed to a mixture of nitric oxide (1.7 ppm) and nitrogen dioxide (0.14 ppm) for 7 years there was an elevated incidence of interalveolar pores and a tendency to changes compatible with emphysema, but the latter changes were not statistically proven (18).

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Consensus Report for Nitrogen Dioxide

September 12, 2007

This document is based primarily on surveys from DECOS (14) and IPCS (38). Supplementary material was obtained in a literature search made in December of 2005, and some information was obtained from an American survey article (18). The present document updates the Consensus Report published by the Criteria Group in 1986 (49).

Chemical and physical data

CAS No.:	10102-44-0
EC No.:	007-002-00-0
Synonym:	nitrogen peroxide
Molecular weight:	46.01
Formula:	NO_2
Melting point:	- 9.3°C
Boiling point:	21.2°C
Vapor pressure:	96 kPa (20°C)
Relative density:	1.58 (air = 1)
Conversion factors:	1 mg/m^3 = 0.52 ppm
	1 ppm = 1.91 mg/m^3

Nitrogen dioxide in liquid phase is yellow, and in gas phase is a reddish-brown gas that is strongly oxidizing. It has a sweetish odor and an odor threshold of 0.1 to 0.4 ppm. At 160°C it disintegrates into nitric oxide (NO) and oxygen.

Occurrence in work environments

Nitrogen dioxide is formed when the nitrogen in the air is oxidized by electrical discharges (including lightning strikes) or combustion, notably in internal combustion engines and around welding. It can also be produced by volcanic activity. These processes create mostly NO, which is then oxidized to nitrogen dioxide by the oxygen and ozone in the air. Nitrogen dioxide occurs in the general environment in concentrations around 0.01 – 0.05 ppm. In Sweden the major source is exhaust from diesel engines (55). Nitrogen dioxide is also formed during combustion of bottled gas and city gas for cooking and heating, and in vehicles run on LPG (liquefied petroleum gas). In indoor arenas where LPG-powered ice machines are used, nitrogen dioxide levels are commonly around 0.1 – 0.8 ppm with peaks approaching 3 – 4 ppm, but considerably higher concentrations have

been measured under special circumstances (64). Tobacco smoking can also contribute to the occurrence of nitrogen dioxide.

Occupational exposures to very high levels of nitrogen dioxide can occur in enclosed areas, notably with gas welding, work in fodder silos, and dynamiting in mines. The most common source of exposure is exhaust from diesel engines, particularly in enclosed, poorly ventilated locations such as tunnels and mines.

For professional drivers in city traffic, exposure levels can exceed urban background levels. For drivers of buses, trucks and taxis in Stockholm in the late 1990s, the average exposure during a workday was 0.014 – 0.016 ppm (0.026 – 0.030 mg/m^3) (47). Exposure averages ranged from 0.005 to 0.03 ppm. In a study of Paris taxi drivers, the average level in the vehicle was 0.07 ppm and the background level was 0.04 ppm. In Norway, levels from 0.4 to 0.9 ppm have been recorded in underground construction projects, but higher peaks (9.9 ppm) were recorded when workers passed areas where dynamite had been used (14). An average level of 0.15 ppm was recently measured for 18 miners in a Swedish iron mine (personal monitors: range 0.03 – 0.35 ppm) (75).

Uptake, biotransformation, excretion

Nitrogen dioxide in exposure situations is almost always in gas form, and the gas can be deposited and absorbed at any level in the respiratory passages. Healthy subjects absorbed 75 – 90% of inhaled nitrogen dioxide. On contact with water, nitrogen dioxide can form nitric acid and nitrous acid, which in turn can decompose, forming nitrite and nitrate ions. No data on skin absorption were found (14). Nitrite in the body can be reduced to nitric oxide, which is a signal molecule (48).

Absorbed nitrogen dioxide is distributed throughout the body in blood. Very little is known about biotransformation or toxicologic mechanisms, but it is assumed that nitrogen dioxide exerts its toxicity partly by its strong oxidizing effect (14). Elimination occurs by biotransformation to nitrate, which is excreted in urine. No data were found on half times in human blood or urine. However, most of the nitrate excreted in urine comes from intake of nitrates and nitrites in food.

Nitrite salts formed from nitrogen dioxide can be oxidized to nitrates. Nitrogen dioxide can bind to unsaturated fats and proteins, e.g. in cell membranes. This may contribute to changes in cell membranes and be one of the mechanisms behind nitrogen dioxide's toxicity (14).

No data on human excretion were found. In experiments with rats, it was found that nitrogen dioxide and its breakdown products are excreted as nitrate in urine. When rats were exposed to low levels of nitrogen dioxide for 24 hours and then monitored for 3 days, the total excretion of nitrate showed a linear correlation to air concentration (62). Using nitrate in urine as a measure of human exposure to airborne nitrogen dioxide is complicated by the fact that people ingest large amounts of nitrate in food.

Toxic effects

Human data

There are several case reports of severe poisoning by nitrogen dioxide. Most of them are due to accidents with nitric acid or exposure in fodder silos. High exposure has led to severe poisoning with acute pulmonary edema and massive inflammation of airways. It also happens that persons exposed to high levels develop severe and sometimes fatal bronchiolitis (inflammation of small airways) after a couple of weeks, even though symptoms were initially moderate (13, 19). The reports on these accidents contain no information on exposure levels.

Gases from ice machines have caused at least seven exposure incidents in Swedish hockey arenas since the mid-1990s. Usual symptoms were coughing, breathlessness and chest pains. Nitrogen dioxide levels during these incidents, derived by duplicating the conditions as closely as possible, were probably 1 to 11 ppm. Symptoms due to carbon monoxide poisoning also occurred in several cases (64). A five-year follow-up of persons exposed to a high level of nitrogen dioxide in an indoor hockey rink (estimated level 1.3 ppm) revealed an elevated occurrence of upper respiratory symptoms five years after the exposure (57).

Several studies in which people were briefly exposed to nitrogen dioxide are reviewed below. Subjects have usually been described as healthy, asthmatic, or persons with chronic obstructive pulmonary disease (COPD). There are no studies covering effects of long-term exposures. Existing epidemiological studies cover exposure to mixed air pollutants, such as diesel exhaust, where nitrogen dioxide is only one of several components. Nitrogen dioxide is sometimes used as a marker for “air pollution” but it is not at all clear whether the described effects are due to nitrogen dioxide.

Effects of short-term exposure on healthy persons

In studies with healthy volunteers exposed for from 10 – 20 minutes up to a few hours on a single day or on only a few occasions, effects examined are those on

– lung function, including bronchial hyperreactivity
– inflammatory markers
– immune defense

Occurrence of symptoms is seldom mentioned in these studies, but they seem to appear at higher doses than the effects listed above. There are many of these studies. (Individual studies are presented in more detail in References 14, 18, 38 and 73.)

According to the WHO document published in 2000, healthy subjects were seldom affected by short-term exposure to levels below 1 ppm, whereas a clear effect in the form of reduced lung function could be demonstrated after exposures above 2.5 ppm (73). DECOS states that effects can be clearly demonstrated at levels above 2 ppm and that the lowest level at which a significant effect is observed is 1.5 ppm, where increased bronchial reactivity can be demonstrated (14, 23). A survey from the California Environmental Protection Agency con-

cludes that definite effects on airway function do not appear until around 4 ppm although effects on inflammatory markers can be seen at about 1.5 to 2 ppm (18). A few of the studies on which these conclusions are based are described below.

Twenty healthy volunteers were exposed to 0.2, 0.4 or 0.8 ppm nitrogen dioxide for 2 hours, 1 day/week for 4 weeks. No significant changes were observed in lung function, bronchial reactivity, alveolar permeability or nasal mucociliary clearance (56, cited in Reference 14).

Five healthy volunteers, all nonsmokers, were exposed to 0.6 ppm nitrogen dioxide 2 hour/day for 4 days: there was no increase in symptoms and there were no effects on lung function or changes in lymphocyte levels in either blood or bronchoalveolar lavage (BAL) (58).

Fifteen healthy subjects were exposed to 1.5 ppm nitrogen dioxide for 3 hours: bronchial reactivity was significantly higher after the exposure. No effect was observed when these subjects were exposed for 3 hours to 0.6 ppm (26).

Frampton *et al.* examined 21 healthy persons exposed to nitrogen dioxide (0.6 or 1.5 ppm) for 3 hours with periods of moderate exercise. After the exposure there was a significant trend to lower numbers of red blood cells and circulating lymphocytes in blood with increasing level of nitrogen dioxide. A phenotype analysis of the lymphocytes ($CD4^+$, $CD8^+$, $CD16^+$, NK cells) revealed no significant correlation to nitrogen dioxide level. There was no significant effect on the total number of cells in bronchial or alveolar lavage. With increasing nitrogen dioxide level there were increases in neutrophilic granulocytes in both alveolar and bronchial lavage, but the lymphocyte content was higher at 0.6 ppm than at 1.5 ppm. However, a phenotyping showed that the level of $CD4^+$ lymphocytes in alveolar lavage increased with increasing nitrogen dioxide. These results are hard to interpret, since with the method of statistical analysis used (variation analysis) it cannot be determined whether significant effects appeared at 0.6 ppm or only at the higher dose of 1.5 ppm. In addition, it was shown that epithelial cells from bronchial mucosa of persons exposed to 1.5 ppm liberated more lactate dehydrogenase after provocation with a virus, although no change in susceptibility to viral infection was seen (measured as RSV virus infection *in vitro*). There were no observed effects on lung function (FVC, FEV_1, sGaw) (21).

Eighteen healthy nonsmokers had higher bronchial reactivity after 1 hour of exposure to 2 ppm nitrogen dioxide (52). Otherwise there were no effects, either on occurrence of symptoms or on lung function (FEV_1, VC).

Fifteen persons (4 women and 11 men) were exposed for 6 hours to either 2 ppm nitrogen dioxide or pure air. The exposures were made twice, with 12 persons in each session. BAL was taken either directly after the exposure or 18 hours later. Lung function, bronchial reactivity and symptoms were studied. There were higher levels of neutrophilic granulocytes in BAL 18 hours after the NO_2 exposure, but no changes in lung function, bronchial reactivity or symptoms (4).

Blomberg *et al.* exposed 12 healthy subjects to 2 ppm for 4 hours on 4 consecutive days (11). After the first day of exposure there was a significant decline in lung function, but the effect became less pronounced on the following

days. After the final exposure, inflammatory changes were assessed by biopsies and bronchial lavage fluid and compared with the results of exposure to pure air. The number of neutrophilic leukocytes in bronchial mucosa was higher after exposure to nitrogen dioxide. The total number of cells in the bronchial lavage fluid was not affected, but certain cell types increased in number ($CD25^+$ lymphocytes and $HLA\text{-}DR^+$ macrophages) while others did not ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16^+56^+$, $CD45RO^+$, $CD69^+$). Myeloperoxidase in bronchial lavage fluid increased after the nitrogen dioxide exposure, but the protein level decreased. Bronchoalveolar lavage fluid was unchanged by the exposure.

Eighteen healthy nonsmokers were exposed to three different concentrations of nitrogen dioxide (2.25, 4 or 5.5 ppm) for 20 minutes, and changes in bronchoalveolar lavage fluid and lung function were then studied (61). The number of mast cells was seen to increase at all exposures, and the number of lymphocytes was increased at the two higher doses. No changes in lung function were found. The authors concluded that brief exposure to these doses caused an inflammatory reaction in respiratory passages.

In a randomized experiment, healthy subjects were exposed to an influenza virus together with nitrogen dioxide (1, 2 or 3 ppm) or air. There were no statistically proven differences, but according to the authors exposure to 1 to 2 ppm nitrogen dioxide may reduce resistance to infection (29).

Experimental short-term exposure of subjects with mild asthma

DECOS reports several studies in which asthmatics were exposed to nitrogen dioxide with or without an allergen, but presents no critical summary. WHO points out that some, but not all, studies show an effect with increased bronchial reactivity at levels of 0.2 to 0.3 ppm (73). A survey from the California Environmental Protection Agency concludes that at exposure to 0.25 ppm there is a statistically proven increase in the effect of allergens on airway function. It is also mentioned that there are studies indicating that exposures of 0.1 to 0.3 ppm increase bronchial reactivity, but that these studies are neither consistent in results nor properly described (18). Some of the studies supporting these conclusions are described below.

In 1992 Folinsbee made a meta-analysis of 20 studies of asthmatics, and concluded that there were indications of increased bronchial reactivity at doses as low as 0.1 to 0.2 ppm (20). He found no dose-response relationship, and a relationship was seen only in studies in which the subjects were resting during the exposure. If asthmatics were exposed during exercise, no effects were observed even in studies with exposures exceeding 0.3 ppm. His conclusions have been criticized, since his analysis is based partly on studies not published in journals using a peer review system (18). The author himself expresses some reservation about attributing the reported effects to nitrogen dioxide.

There are several studies of asthmatics in which lung function was studied with brief experimental exposure to nitrogen dioxide at levels around 0.4 ppm or below. Three studies reporting effects on lung function at 0.3 ppm are presented

in Table 2 (3, 7, 44), but there are several other studies reporting no such effects. (A survey is presented in Reference 18.)

Bylin *et al*. exposed 8 healthy persons and 8 asthmatics to 0 (controls), 0.12, 0.24, or 0.48 ppm nitrogen dioxide for 20 minutes on four different occasions (12). In the healthy subjects airway resistance increased at 0.24 ppm but decreased at 0.48 ppm, which is a finding difficult to interpret. In the asthmatics, bronchial reactivity increased (histamine test) and thoracic gas volume (TGV) decreased after exposure to 0.48 ppm.

Nineteen persons with mild asthma were exposed for 30 minutes to either air (controls) or 0.26 ppm nitrogen dioxide, and changes in bronchial reactivity, airway resistance and TGV were measured 30 minutes, 5 hours, 7 hours and 7 days later. Blood samples were taken 30 minutes and 27 hours after the exposure. With the nitrogen dioxide exposure there was a significant increase in bronchial reactivity at 5 hours, and a trend to increase at 30 minutes. In the blood samples taken at 30 minutes there was an increase of Mac-1 on granulocytes, but the other blood samples showed no changes. This observation is hard to interpret, since the significance of changes in this marker is unclear (67).

Strand *et al.* examined 16 persons with mild asthma and allergy to birch or grass pollen. On four consecutive days they were exposed at random to either air or 0.26 ppm nitrogen dioxide for 30 minutes, followed four hours later by a nonsymptomatic allergen dose (68). A reduction in lung function (FEV_1) and a tendency to increased nocturnal symptoms were seen with the exposure to nitrogen dioxide + allergen, compared with the exposure to air + allergen.

A similar experiment made by Barck *et al.* (0.26 ppm for 30 minutes followed by allergen, 13 persons with mild asthma) reports elevated occurrence of neutrophilic leukocytes and eosinophilic cationic protein (ECP) in bronchial lavage fluid after exposure to nitrogen dioxide plus allergen, compared to air plus allergen. There were no effects on lung function or occurrence of symptoms (6).

Eighteen persons with mild asthma were exposed to 0.26 ppm nitrogen dioxide for 15 minutes on day 1 and 2 x 15 minutes on day 2, followed on both days by allergen exposure. They had higher levels of ECP in sputum and blood than when they were exposed on the same schedule to air and allergen (5). Myeloperoxidase was elevated in blood but not in sputum. No changes in lung function or occurrence of symptoms were observed.

Jenkins *et al.* exposed 11 subjects with mild allergic asthma to 0.2 (6 hours) or 0.4 (3 hours) ppm nitrogen dioxide, followed by allergen exposure. They found no effect on bronchial reactivity (measured as the dose of allergen necessary to reduce FEV_1 by 20%) at 0.2 ppm, but a significant effect at 0.4 ppm (41).

Ten persons with mild allergic asthma were exposed on different occasions to 0, 0.1 or 0.4 ppm nitrogen dioxide and immediately thereafter provoked with an allergen (mite dust). At the higher dose FEV_1 was significantly lower than with exposure to air and mite dust (70).

Effects of short-term exposures on persons with chronic obstructive pulmonary disease (COPD)

In studies of healthy persons and asthmatics the subjects are generally young, whereas in studies of persons with chronic obstructive pulmonary disease the subjects are older, since COPD is primarily a disease of older people. In the view of the California Environmental Protection Agency, the results of published studies are not wholly consistent but the differences may be due to differences in sensitivity between the studied groups (18). Some of the studies on which this view is based are presented below.

In a randomized experiment, 20 persons with COPD (13 men and 7 women, average age 60) and 20 healthy persons inhaled either 0.3 ppm nitrogen dioxide or pure air for four hours. For those with COPD there was a statistically significant reduction of FEV_1 after the nitrogen dioxide exposure (54).

Eighteen persons with COPD (average age 61) inhaled 0.4 ppm nitrogen dioxide for 2 hours and no effect on either symptoms or lung function was found (30).

Studies of nitrogen dioxide in the general environment

Correlations between health and nitrogen dioxide in air have been made in several studies in which nitrogen dioxide was one of several air pollutants in the general environment. These studies show correlations between variations in nitrogen dioxide levels in air within a city and mortality, as well as elevations in risk for death due to heart and circulatory disease, lung disease and cancer, when cities with different average levels of nitrogen dioxide were compared. Such studies have identified effects at levels that occur in Swedish cities. Air pollutants have also been shown to affect lung development in young people (28). Nitrogen dioxide level has also been used as an indicator for diesel exhaust and its effects on health. Since it is impossible to determine what is an effect of nitrogen dioxide and what is an effect of other pollutants co-varying with nitrogen dioxide, no conclusions on health effects from nitrogen dioxide can be drawn from these studies. Average levels of nitrogen dioxide in Stockholm, Gothenburg and Malmö are around 0.02 – 0.04 mg/m^3 (0.01 – 0.02 ppm) (55). In a German survey it is pointed out that, in short-term experimental exposures, effects of nitrogen dioxide are seen in humans at 0.4 mg/m^3 (0.2 ppm) but not at 0.2 mg/m^3 (0.1 ppm) (45). It is also pointed out that there are indications that the etiology of emphysema may be connected to nitrogen dioxide and that epidemiological studies have not provided evidence of a limit below which long-term exposure does not constitute a risk to health.

The risks of nitrogen dioxide exposure have also been discussed in connection with gas stoves. Food preparation can be associated with nitrogen dioxide levels up to 1 ppm (15), as well as nitrous oxide (up to 2 ppm) and particles. Studies of the health risks of nitrogen dioxide associated with use of gas stoves for cooking are not consistent, but it is possible that sensitive persons may be affected (40).

Animal data

Brief (2 – 5 minutes), high exposures (955 to 3820 mg/m^3, 500 to 2000 ppm) have caused pulmonary edema and death in rats and mice (14).

Three hours of exposure to 3 ppm together with a bacterial aerosol increased mortality in mice (37). Exposure to 3 ppm for 24 hours affected mucociliary transport in rabbits (42).

There are several studies in which animals were exposed for weeks or months to nitrogen dioxide, sometimes with virus or bacteria, to study sensitivity to infection. The DECOS survey reviews studies in which animals were exposed to 0.06 – 2 ppm (14). DECOS interprets these data as indicating that at levels above 0.5 ppm there are consistently observed effects in airways – increased vulnerability to infection, changes in biochemical markers, morphological changes etc. – and that apparently the effects are sometimes reversible. A few studies have examined effects of exposures below 0.5 ppm, and these are presented below.

Kobayashi *et al.* (43) studied guinea pigs exposed to nitrogen dioxide for 6 to 12 weeks. Concentrations were 0.06, 0.5, 1, 2 or 4 ppm. They found effects on lung function at 1 ppm and higher.

Ehrlich *et al.* (16) exposed mice to 0.1 ppm nitrogen dioxide for one to six months. They were also exposed for 3 hours/day, 5 days/week to an additional 0.5 ppm. Their final nitrogen dioxide exposure was followed one hour later by a bacterial aerosol. Mortality was elevated during some periods, and the authors regard this as increased sensitivity to infection.

Mice were exposed to 0.34 ppm nitrogen dioxide 6 hours/day, 5 days/week for 6 weeks. Quantitative image analysis revealed increases in the number and size of Type II alveolar cells and indications of possible edema in alveolar walls (63).

Mice exposed to 0.5 ppm nitrogen dioxide for 3 to 12 months (6 to 24 hours/day) developed changes in airways compatible with emphysema. The changes were most pronounced in the animals with most exposure (10).

There are several published studies in which animals were exposed to nitrogen dioxide for more than 6 months. All the studies report deviations from normal in animals exposed to 0.5 ppm or more for at least 12 months – lower survival, reduced immune defense, changes in lung morphology etc. (14).

Rats were exposed to 0.04, 0.4 or 4 ppm and monitored for 9 to 27 months (46, 59, 60). No effects were observed at the lowest exposure level. At the two higher doses there were effects on enzyme activity, including glutathione transferase. At the highest dose there was also some interstitial fibrosis, but alveolar structure was not affected. These effects could also be confirmed by electron microscopy at 0.4 ppm, but they were generally weaker and appeared later than at 4 ppm.

Dogs were exposed to mixtures of nitrogen dioxide and nitric oxide (either 0.27 mg/m^3 NO_2 + 2.05 mg/m^3 NO or 1.2 mg/m^3 NO_2 + 0.31 mg/m^3 NO) 16 hours/day for 7 years, and then to pure air for 32 to 36 months (35). The two-gas mixtures were used to resemble air pollution that can occur in the general environment. The dogs exposed to the higher dose of nitrogen dioxide (1.2 mg/m^3 = 0.62 ppm) had significantly larger lungs and indications of destruction of alveolar walls; cilia loss

and bronchial metaplasias were also seen in this group. The changes in the animals exposed to the lower dose (0.27 mg/m^3 = 0.14 ppm) were less pronounced: aside from an increase in the number of interalveolar pores, there were no statistically significant differences from controls. The authors regard the changes observed at the higher dose of nitrogen dioxide as early indications of emphysema. It is difficult to determine whether nitric oxide is potentiating.

Mice were exposed to 0.2 ppm nitrogen dioxide for 16 – 52 weeks and then provoked with bacteria. There were no differences from controls (51).

Rats were exposed to 0.5 ppm for up to 19 months; there were changes in bronchioles (interstitial edema and thickened alveolar walls, swelling of type II alveolar cells) compared to controls. There was also increased fibrosis in pleura, but not in lung tissue (33).

Mice were continuously exposed to 0.1 ppm plus 0.5 ppm 3 hours/day, 5 days/week, or to 0.5 ppm 3 hours/day, 5 days/week without the background exposure, for 1 to 6 months, and then exposed to bacteria. There was a significant increase in mortality after six months of exposure (17).

Mutagenicity, genotoxicity, carcinogenicity

The results of genotoxicity studies are both inconsistent and hard to interpret (14). *In vitro* studies indicate that nitrogen dioxide may be genotoxic. Studies with Drosophila do not show evidence of genotoxicity. Four *in vivo* studies report no increase in genotoxicity (mice: 0.2, 1.9, 9.5 or 19.1 mg/m^3 (31); rats: 2.26 mg/m^3 (9); mice: 38.2 mg/m^3 (74); Drosophila: 95.5 mg/m^3 (74)), while two report increased genotoxicity (rats: 15.3 – 53.3 mg/m^3 (39); mice: 57.3 or 95.5 mg/m^3 (72)). It has been suggested that the differences in results may be due to the fact that nitrogen dioxide can form other compounds that are genotoxic (e.g. nitrosamines), and that the method of administration may be relevant.

No studies were found on cancer risk for humans.

No increase in tumor incidence was seen in rats exposed to 0.04, 0.4 or 4 ppm nitrogen dioxide for 27 months (46). Nor was any increase seen in rats exposed to 9.5 ppm for 2 years (50). In a study in which several species – rats, mice, hamsters, guinea pigs, rabbits and dogs – were exposed to 1, 5 or 25 ppm for 10 to 18 months, no definite elevation in cancer risk was seen (71). None of these studies was designed to study cancer risk; they were all focused on other effects.

A strain of mice with a high frequency of spontaneous lung tumors showed a significant increase of lung tumors after 6 months of exposure to 10 ppm (compared with a pooled control group), but no effect was seen at 1 or 5 ppm (2).

To determine whether nitrogen dioxide might function as a promoter, rats were injected with a known tumor-causing substance (N-bis(2-hydroxypropyl)nitrosamine). There was no statistically significant effect on tumor frequency at exposures of 0.04, 0.4, or 4 ppm for 17 months. With reference to the slightly higher number of tumors at the highest dose (4/40, compared with 1/40 in controls), the authors nevertheless conclude that the study indicates that nitrogen dioxide at this level has a tumor-promoting effect (36).

According to DECOS, none of these animal studies meets present quality standards for carcinogenicity tests (14).

Nitrogen dioxide has not been classified by the IARC.

Effects on reproduction

DECOS reports three studies, and states that due to their poor quality no conclusions on reproduction toxicity can be drawn from them (14).

Dose-effect / dose-response relationships

Nitrogen dioxide almost always occurs together with other nitrogen oxides, in the work environment as well as the general environment, and studies are therefore very difficult to evaluate with regard to the contribution made by nitrogen dioxide to the observed effects.

For humans, there are experimental studies only with short-term exposures, but in compensation there are several of these. Table 1 presents a selection of studies with healthy volunteers and the levels at which the critical effect was observed. Table 2 presents the same information for persons with mild asthma.

With *short-term* exposure of *healthy* subjects, effects on lung function begin to appear at levels around 1.5 – 2 ppm, but results vary somewhat. At these levels there are also indications that inflammatory markers in the lungs increase (see Table 1). One study reports effects on inflammatory markers at 0.6 – 1.5 ppm, but was made in such a way that it can not be determined whether the effects were seen only at the higher level or at both levels (21). A study of hockey players involved in incidents of nitrogen dioxide poisoning in hockey arenas revealed a higher incidence of upper respiratory symptoms 5 years later (57). The nitrogen dioxide levels during the exposure incidents are not known, but duplication of conditions yielded levels up to 11 ppm. The victims had been physically active in connection with the incidents.

Persons with *mild asthma* were found in three studies to have reduced lung function at 0.3 ppm, but other studies report no such effects. Increased bronchial reactivity at levels around 0.2 – 0.3 ppm has been demonstrated in some studies. With simultaneous exposure to an allergen and 0.25 ppm nitrogen dioxide, effects on lung function, bronchial reactivity and inflammatory markers have been shown in sensitized asthmatics (see Table 2). Over 5% of the adult working population has asthma (53).

In one study, of subjects in their 60s who had chronic obstructive pulmonary disease (COPD), transient effects on lung function were seen after 4 hours of exposure to 0.3 ppm (54).

There are no studies of *long-term exposure* of humans.

If nitrogen dioxide is used as an indicator substance for air pollution in the general environment, effects on morbidity can be seen at levels occurring in Swedish metropolitan areas (average 0.01 – 0.02 ppm).

Table 1. A representative selection of studies in which healthy subjects were briefly exposed to nitrogen dioxide. (For more information, see References 14 and 18.)

Level (ppm)	Time	Number of subjects*	Effects	Ref.
0.6	1 hour	40 (20/20)	No effect on lung function.	1, 14
0.6	3 hours	9 (2/7)	No effect on lung function or bronchial reactivity. No significant effects on inflammatory markers (BAL).	22, 23
0.6	3 hours	21 (9/12)	No effect on lung function. No effect on vulnerability to infection measured *in vitro*.	21
1	2 hours	16	No effect on lung function.	8
1	2 hours x 2 days	15	No effect on lung function.	32
1	2 hours x 3 days	21	No effect on lung function or bronchial reactivity. No increase in sensitivity to infection.	29
1.5	3 hours	15 (3/12)	No changes in lung function. Increased reactivity in airways compared with controls. No inflammatory changes in BAL.	22, 23
0.6 – 1.5	3 hours	21 (9/12)	No effect on lung function. Increase of inflammatory markers in BAL.	21
2	2 hours x 3 days	21	No effect on lung function or bronchial reactivity. No increase in sensitivity to infection.	29
2	1 hour	18 (5/13)	Increased reactivity in airways.	52
2	6 hours	12	Increase of neutrophilic granulocytes in BAL.	14, 24
2	4 hours x 4 days	12 (4/8)	Effects on lung function on day 1. Slight indications of inflammation at end of exposure.	11
2.25	20 min.	8	Increase of mast cells in BAL.	14, 61
3	2 hours x 3 days	21	No effect on lung function or bronchial reactivity. No increase in sensitivity to infection.	29

* (women/men). Some studies do not provide this information.

Effects at the alveolar level (suspected edema and cell proliferation) have been documented in mice exposed to 0.34 ppm for 6 weeks (63). Mice continuously exposed to 0.1 ppm with additional intermittent exposures of 0.5 ppm were more vulnerable to infection than controls (16). In other animal experiments, inflammatory changes in lungs and indications of increased vulnerability to infection have been observed at 0.5 ppm (Table 3) (17, 33). Mice exposed to 0.5 ppm for 12 months had indications of emphysema (10). Dogs exposed to 0.6 ppm nitrogen dioxide (plus 0.3 ppm nitric oxide) for 7 years had slight emphysema-like changes in lungs and loss of cilia in airways (35). Anatomical conditions and

mathematical models based on them (69) indicate, however, that the quantitative aspects of these data can not be directly extrapolated to humans.

Conclusions

In laboratory studies with human subjects, the critical effect of short-term exposure to nitrogen dioxide is its effect on respiratory passages in the form of increases in bronchial reactivity and inflammatory markers. These effects can be seen in healthy subjects at levels around 1.5 ppm.

Persons with mild asthma or chronic obstructive pulmonary disease can show effects on lung function at levels around 0.3 ppm. This probably includes at least 5% of the working population.

Table 2. Studies of subjects with mild asthma given short-term exposures to nitrogen dioxide, with or without subsequent administration of an allergen. (These studies are described in greater detail in References 14 and 18.)

Level (ppm)	Time	Allergen	Effects	Ref.
0.12	20 min.	No	No effect on lung function.	12
0.24	20 min.	No	No effect on lung function.	12
0.26	30 min. x 4 days	No	No effect on lung function.	68
0.26	30 min.	No	Increased bronchial reactivity.	67
0.3	30 min.	No	Increased bronchial reactivity and transient reduction in FEV_1	7
0.3	3 hours	No	No effect on bronchial reactivity, but reduced lung function after 1 hour and improved lung function after 3 hours of exposure.	3
0.3	30 min.	No	Slight, temporary reduction of FVC.	44
0.47	20 min.	No	Increased bronchial reactivity.	12
0.1	1 hour	Yes	No effect on lung function.	70
0.2	6 hours	Yes	No effect on lung function.	41
0.26	30 min. x 4 days	Yes	Temporary decline in lung function, tendency to increased nocturnal symptoms.	68
0.26	30 min.	Yes	Increase of inflammatory markers in respiratory passages.	6
0.26	15 min. day 1 + 30 min. day 2	Yes	Increase of inflammatory markers in respiratory passages.	5
0.4	1 hour	Yes	Effects on lung function.	70
0.4	6 hours	Yes	Effects on lung function.	41

Table 3. Morphological effects observed in the lungs of animals after long-term exposure to nitrogen dioxide. (See also References 14 and 18.)

Level (ppm)	Time	Species	Effects	Ref.
0.04	27 mos.	Rat	None.	46
0.14 (+ 1.7 ppm NO)	7 years	Dog	Slight changes compatible with emphysema (not statistically significant).	35
0.2	12 mos.	Mouse	None.	51
0.34	6 weeks	Mouse	Increases in number and size of Type II alveolar cells, possibly edema of alveolar walls.	63
0.4	27 mos.	Rat	Difficult to interpret.	46
0.1 (back-ground) + 0.5 for 3 h/day	1 to 6 mos.	Mouse	Increased vulnerability to infection.	16
0.5	12 mos.	Mouse	Indications of increased vulnerability to infection (pneumonitis) after 3 and 6 months.	17
0.5	19 mos.	Rat	Indications of inflammation: interstitial edema, increase of alveolar septa, fibrous thickening of pleura.	33
0.6 (+ 0.3 ppm NO)	7 years	Dog	Indications of slight emphysema, loss of cilia in respiratory passages.	35
0.8	up to 33 mos.	Rat	Minimal changes.	25*, 34*
1	18 mos.	Guinea pig	Slight thickening of alveolar septa.	71*
2	2 years	Rat	Changes in cilia of terminal bronchioles.	65*, 66*
2	14 mos.	Rat	Minimal effect, slight hypertrophy of epithelium in terminal bronchioles.	27*
2	up to 763 days	Rat	Expansion of alveoli, cilia loss and hypertrophy in terminal bronchioles.	26*
4	16 mos.	Rat	Hyperplasia of bronchial epithelium.	34*
4	27 mos.	Rat	Fibrosis.	46

* Cited in Reference 14.

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Summary

Montelius J (ed). *Scientific Basis for Swedish Occupational Standards.* XXVIII. Arbete och Hälsa 2008:42:6, 92 pp. Swedish Work Environmental Authority, Solna.

Critical review and evaluation of those scientific data which are relevant as a background for discussion of Swedish occupational exposure limits. This volume consists of the consensus reports given by the Criteria Group at the Swedish Work Environmental Authority from July, 2006 through September, 2007.

Key Words: Nitric oxide, Nitrogen dioxide, Occupational exposure limit (OEL), Ozone, Risk assessment, Scientific basis, Toxicology, White spirit.

Sammanfattning

Montelius J (ed). *Vetenskapligt underlag för hygieniska gränsvärden.* XXVIII. Arbete och Hälsa 2008:42:6, 92 pp. Arbetsmiljöverket, Solna.

Sammanställningar baserade på kritisk genomgång och värdering av de vetenskapliga fakta, vilka är relevanta som underlag för fastställande av hygieniskt gränsvärde. Volymen omfattar de underlag som avgivits från Kriteriegruppen för hygieniska gränsvärden under perioden juli 2006 – september 2007.

Nyckelord: Hygieniskt gränsvärde, Kväveoxid, Kvävedioxid, Lacknafta, Ozon, Riskvärdering, Toxikologi, Vetenskapligt underlag.

En svensk version av dessa vetenskapliga underlag, med undantag för lacknafta, finns publicerad i Arbete och Hälsa 2008;42:3. Den svenska versionen av lacknafta är publicerad i Arbete och Hälsa 2006:9.

APPENDIX

Consensus reports in this and previous volumes

Substance	Consensus date	Published in Arbete och Hälsa (year;volume:No.)
Acetaldehyde	February 17, 1987	1987:39
Acetamide	December 11, 1991	1992:47
Acetic acid	June15, 1988	1988:32
Acetone	October 20, 1987	1988:32
Acetonitrile	September 12, 1989	1991:8
Acrylamide	April 17, 1991	1992:6
Acrylates	December 9, 1984	1985:32
Acrylonitrile	April 28, 1987	1987:39
Aliphatic amines	August 25, 1982	1983:36
Aliphatic hydrocarbons, C10-C15	June 1, 1983	1983:36
Aliphatic monoketons	September 5, 1990	1992:6
Allyl alcohol	September 9, 1986	1987:39
Allylamine	August 25, 1982	1983:36
Allyl chloride	June 6, 1989	1989:32
Aluminum	April 21, 1982	1982:24
revised	September 14, 1994	1995:19
Aluminum trifluoride	September 15, 2004	2005:17
p-Aminoazobenzene	February 29, 1980	1981:21
Ammonia	April 28, 1987	1987:39
revised	October 24, 2005	2006:11
Ammonium fluoride	September 15, 2004	2005:17
Amylacetate	March 23, 1983	1983:36
revised	June 14, 2000	2000:22
Aniline	October 26, 1988	1989:32
Anthraquinone	November 26, 1987	1988:32
Antimony + compounds	December 8, 1999	2000:22
Arsenic, inorganic	December 9, 1980	1982:9
revised	February 15, 1984	1984:44
Arsine	October 20, 1987	1988:32
Asbestos	October 21, 1981	1982:24
Barium	June 16, 1987	1987:39
revised	January 26, 1994	1994:30
Benzene	March 4, 1981	1982:9
revised	February 24, 1988	1988:32
Benzoyl peroxide	February 13, 1985	1985:32
Beryllium	April 25, 1984	1984:44
Borax	October 6, 1982	1983:36
Boric acid	October 6, 1982	1983:36
Boron Nitride	January 27, 1993	1993:37
Butadiene	October 23, 1985	1986:35
1-Butanol	June 17, 1981	1982:24

Butanols	June 6,	1984	1984:44
Butyl acetate	June 6,	1984	1984:44
Butyl acetates	February 11,	1998	1998:25
Butylamine	August 25,	1982	1983:36
Butyl glycol	October 6,	1982	1983:36
γ-Butyrolactone	June 2,	2004	2005:7
Cadmium	January 18,	1980	1981:21
revised	February 15,	1984	1984:44
revised	May 13,	1992	1992:47
revised	February 5,	2003	2003:16
Calcium fluorid	September 15,	2004	2005:17
Calcium hydroxide	February 24,	1999	1999:26
Calcium nitride	January 27,	1993	1993:37
Calcium oxide	February 24,	1999	1999:26
Caprolactam	October 31,	1989	1991:8
Carbon monoxide	December 9,	1981	1982:24
Cathecol	September 4,	1991	1992:47
Chlorine	December 9,	1980	1982:9
Chlorine dioxide	December 9,	1980	1982:9
Chlorobenzene	September 16,	1992	1993:37
revised	April 2,	2003	2003:16
o-Chlorobenzylidene malononitrile	June 1,	1994	1994:30
Chlorocresol	December 12,	1990	1992:6
Chlorodifluoromethane	June 2,	1982	1982: 24
Chlorophenols	September 4,	1985	1986:35
Chloroprene	April 16,	1986	1986:35
Chromium	December 14,	1979	1981:21
revised	May 26,	1993	1993:37
revised	May 24,	2000	2000:22
Chromium trioxide	May 24,	2000	2000:22
Coal dust	September 9,	1986	1987:39
Cobalt	October 27,	1982	1983:36
Cobalt and cobalt compounds	October 22,	2003	2005:7
Copper	October 21,	1981	1982:24
Cotton dust	February14,	1986	1986:35
Creosote	October 26,	1988	1989:32
Cresols	February 11,	1998	1998:25
Cumene	June 2,	1982	1982:24
Cyanamid	September 30,	1998	1999:26
Cyanoacrylates	March 5,	1997	1997:25
Cycloalkanes, C5-C15	April 25,	1984	1984:44
Cyclohexanone	March 10,	1982	1982:24
revised	February 24,	1999	1999:26
Cyclohexanone peroxide	February 13,	1985	1985:32
Cyclohexylamine	February 7,	1990	1991:8
Desflurane	May 27,	1998	1998:25
Diacetone alcohol	December 14,	1988	1989:32
Dichlorobenzenes	February 11,	1998	1998:25
1,2-Dibromo-3-chloropropane	May 30,	1979	1981:21
Dichlorodifluoromethane	June 2,	1982	1982:24
1,2-Dichloroethane	February 29,	1980	1981:21

Dichloromethane	February 29,	1980	1981:21
Dicumyl peroxide	February 13,	1985	1985:32
Dicyclopentadiene	March 23,	1994	1994:30
Diesel exhaust	December 4,	2002	2003:16
Diethanolamine	September 4,	1991	1992:47
Diethylamine	August 25,	1982	1983:36
2-Diethylaminoethanol	January 25,	1995	1995:19
Diethylene glycol	September 16,	1992	1993:37
Diethyleneglycol ethylether + acetate	December 11,	1996	1997:25
Diethyleneglycol methylether + acetate	March 13,	1996	1996:25
Diethyleneglycol monobutylether	January 25,	1995	1995:19
Diethylenetriamine	August 25,	1982	1983:36
revised	January 25,	1995	1995:19
Diisocyanates	April 8,	1981	1982:9
revised	April 27,	1988	1988:32
Diisopropylamine	February 7,	1990	1991:8
N,N-Dimethylacetamide	March 23,	1994	1994:30
Dimethyl adipate	December 9,	1998	1999:26
Dimethylamine	December 10,	1997	1998:25
N,N-Dimethylaniline	December 12,	1989	1991:8
Dimethyldisulfide	September 9,	1986	1987:39
Dimethylether	September 14,	1994	1995:19
Dimethylethylamine	June 12,	1991	1992:6
Dimethylformamide	March 23,	1983	1983:36
Dimethyl glutarate	December 9,	1998	1999:26
Dimethylhydrazine	January 27,	1993	1993:37
Dimethyl succinate	December 9,	1998	1999:26
Dimethylsulfide	September 9,	1986	1987:39
Dimethylsulfoxide, DMSO	December 11,	1991	1992:47
Dioxane	August 25,	1982	1983:36
revised	March 4,	1992	1992:47
Diphenylamine	January 25,	1995	1995:19
4,4'-Diphenylmethanediisocyanate (MDI)	April 8,	1981	1982:9
revised	May 30,	2001	2001:20
Dipropylene glycol	May 26,	1993	1993:37
Dipropyleneglycol monomethylether	December 12,	1990	1992:6
Disulfiram	October 31,	1989	1991:8
Enzymes, industrial	June 5,	1996	1996:25
Ethanol	May 30,	1990	1991:8
Ethanolamine	September 4,	1991	1992:47
Ethylacetate	March 28,	1990	1991:8
Ethylamine	August 25,	1982	1983:36
Ethylamylketone	September 5,	1990	1992:6
Ethylbenzene	December 16,	1986	1987:39
Ethylchloride	December 11,	1991	1992:47
Ethylene	December 11,	1996	1997:25
Ethylene chloride	February 29,	1980	1981:21
Ethylene diamine	August 25,	1982	1983:36
Ethylene glycol	October 21,	1981	1982:24
Ethylene glycol methylether + acetate	June 2,	1999	1999:26
Ethyleneglycol monoisopropylether	November 16,	1994	1995:19
Ethyleneglycol monopropylether + acetate	September 15,	1993	1994:30

Ethylene oxide	December 9,	1981	1982:24
Ethylenethiourea	September 27,	2000	2001:20
Ethylether	January 27,	1993	1993:37
Ethylglycol	October 6,	1982	1983:36
Ferbam	September 12,	1989	1991:8
Ferric dimethyldithiocarbamate	September 12,	1989	1991:8
Flour dust	December 10,	1997	1998:25
Fluorides	September 15,	2004	2005:17
Formaldehyde	June 30,	1979	1981:21
revised	August 25,	1982	1983:36
Formamide	December 12,	1989	1991:8
Formic acid	June 15,	1988	1988:32
Furfural	April 25,	1984	1984:44
Furfuryl alcohol	February 13,	1985	1985:32
Gallium + Gallium compounds	January 25,	1995	1995:19
Glutaraldehyde	September 30,	1998	1999:26
Glycol ethers	October 6,	1982	1983:36
Glyoxal	September 13,	1996	1996:25
Grain dust	December 14,	1988	1989:32
Graphite	December 10,	1997	1998:25
Halothane	April 25,	1985	1985:32
2-Heptanone	September 5,	1990	1992:6
3-Heptanone	September 5,	1990	1992:6
Hexachloroethane	September 15,	1993	1994:30
Hexamethylenediisocyanate (HDI)	April 8,	1981	1982:9
revised	May 30,	2001	2001:20
Hexamethylenetetramine	August 25,	1982	1983:36
n-Hexanal	March 29,	2006	2006:11
n-Hexane	January 27,	1982	1982:24
2-Hexanone	September 5,	1990	1992:6
Hexyleneglycol	November 17,	1993	1994:30
Hydrazine	May 13,	1992	1992:47
Hydrogen bromide	February 11,	1998	1998:25
Hydrogen cyanide	February 7,	2001	2001:20
Hydrogen fluoride	April 25,	1984	1984:44
revised	September 15,	2004	2005:17
Hydrogen peroxide	April 4,	1989	1989:32
Hydrogen sulfide	May 4,	1983	1983:36
Hydroquinone	October 21,	1989	1991:8
Indium	March 23,	1994	1994:30
Industrial enzymes	June 5,	1996	1996:25
Isocyanic Acid (ICA)	December 5,	2001	2002:19
Isophorone	February 20,	1991	1992:6
Isopropanol	December 9,	1981	1982:24
Isopropylamine	February 7,	1990	1991:8
Isopropylbenzene	June 2,	1982	1982:24
Lactates	March 29,	1995	1995:19
Lactate esters	June 2,	1999	1999:26

Laughing gas	June 7,	2006	2006:11
Lead, inorganic	February 29,	1980	1981:21
revised	September 5,	1990	1992:6
revised	December 8,	2004	2005:17
Lithium and lithium compounds	June 4,	2003	2003:16
Lithium boron nitride	January 27,	1993	1993:37
Lithium nitride	January 27,	1993	1993:37
Maleic anhydride	September 12,	1989	1991:8
Manganese	February 15,	1983	1983:36
revised	April 17,	1991	1992:6
revised	June 4,	1997	1997:25
Man made mineral fibers	March 4,	1981	1982:9
revised	December 1,	1987	1988:32
Mercury, inorganic	April 25,	1984	1984:44
Mesityl oxide	May 4,	1983	1983:36
Metal stearates, some	September 15,	1993	1994:30
Methacrylates	September 12,	1984	1985:32
Methanol	April 25,	1985	1985:32
Methyl acetate	March 28,	1990	1991:8
Methylamine	August 25,	1982	1983:36
Methylamyl alcohol	March 17,	1993	1993:37
Methyl bromide	April 27,	1988	1988:32
Methyl chloride	March 4,	1992	1992:47
Methyl chloroform	March 4,	1981	1982:9
4,4´-methylene-bis-(2-chloroaniline)	February 4,	2004	2005:7
Methylene chloride	February 29,	1980	1981:21
4,4'-Methylene dianiline	June 16,	1987	1987:39
revised	October 3,	2001	2002:19
Methyl ethyl ketone	February 13,	1985	1985:32
Methyl ethyl ketone peroxide	February 13,	1985	1985:32
Methyl formate	December 12,	1989	1991:8
Methyl glycol	October 6,	1982	1983:36
Methyl iodide	June 30,	1979	1981:21
Methylisoamylamine	September 5,	1990	1992:6
Methylisoamylketone	February 6,	2002	2002:19
Methylisocyanate (MIC)	December 5,	2001	2002:19
Methyl mercaptane	September 9,	1986	1987:39
Methyl methacrylate	March 17,	1993	1993:37
Methyl pyrrolidone	June 16,	1987	1987:39
α-Methylstyrene	November 1,	2000	2001:20
Methyl-t-butyl ether	November 26,	1987	1988:32
revised	September 30,	1998	1999:26
Mixed solvents, neurotoxicity	April 25,	1985	1985:32
MOCA	February 4,	2004	2005:7
Molybdenum	October 27,	1982	1983:36
Monochloroacetic acid	February 20,	1991	1992:6
Monochlorobenzene	September 16,	1993	1993:37
Monomethylhydrazine	March 4,	1992	1992:47
Mononitrotoluene	February 20,	1991	1992:6
Monoterpenes	February 17,	1987	1987:39
Morpholine	December 8,	1982	1983:36
revised	June 5,	1996	1996:25

Naphthalene	May 27,	1998	1998:25
Natural crystallinic fibers (except asbestos)	June 12,	1991	1992:6
Nickel	April 21,	1982	1982:24
Nicotine	June 2.	2004	2005:7
Nitric oxide	December 11,	1985	1986:35
revised	June 13,	2007	2008;42:6
Nitroethane	April 4,	1989	1989:32
Nitrogen dioxide	December 11,	1985	1986:35
revised	September 12,	2007	2008;42:6
Nitrogen oxides	December 11,	1985	1986:35
Nitroglycerin	February 13,	1985	1985:32
Nitroglycol	February 13,	1985	1985:32
Nitromethane	January 6,	1989	1989:32
Nitropropane	October 28,	1986	1987:39
2-Nitropropane	March 29,	1995	1995:19
Nitroso compounds	December 12,	1990	1992:6
Nitrosomorpholine	December 8,	1982	1983:36
Nitrotoluene	February 20,	1991	1992:6
Nitrous oxide	December 9,	1981	1982:24
revised	June 7,	2006	2006:11
Oil mist	April 8,	1981	1982:9
Organic acid anhydrides, some	September 12,	1989	1991:8
Oxalic acid	February 24,	1988	1988:32
Ozone	April 28,	1987	1987:39
revised	February 7,	2007	2008;42:6
Paper dust	February 7,	1990	1991:8
Penicillins	November 23,	2005	2006:11
Pentaerythritol	November 16,	1994	1995:19
1,1,1,2,2-Pentafluoroethane	February 24,	1999	1999:26
Pentyl acetate	June 14,	2000	2000:22
Peroxides, organic	February 13,	1985	1985:32
Phenol	February 13,	1985	1985:32
Phosphorous chlorides	September 30,	1998	1999:26
Phosphorous oxides	February 11,	1998	1998:25
Phthalates	December 8,	1982	1983:36
Phthalic anhydride	September 12,	1989	1991:8
Piperazine	September 12,	1984	1985:32
Plastic dusts	December 16,	1986	1987:39
Platinum	June 4,	1997	1997:25
Polyaromatic hydrocarbons	February 15,	1984	1984:44
Polyisocyanates	April 27,	1988	1988:32
Potassium aluminium fluoride	June 4,	1997	1997:25
Potassium cyanide	February 7,	2001	2001:20
Potassium dichromate	May 24,	2000	2000:22
Potassium Fluoride	September 15,	2004	2005:17
Potassium hydroxide	Marsh 15,	2000	2000:22
2-Propanol	December 9,	1981	1982:24
Propene	September 13,	1996	1996:25
Propionic acid	November 26,	1987	1988:32

Propylacetate	September 14,	1994	1995:19
Propylene glycol	June 6,	1984	1984:44
Propylene glycol-1,2-dinitrate	May 4,	1983	1983:36
Propylene glycol monomethylether	October 28,	1986	1987:39
Propylene oxide	June 11,	1986	1986:35
Pyridine	May 13,	1992	1992:47
Quartz	March 13,	1996	1996:25
Resorcinol	September 4,	1991	1992:47
Selenium	December 11,	1985	1986:35
revised	February 22,	1993	1993:37
Sevoflurane	May 27,	1998	1998:25
Silica	March 13,	1996	1996:25
Silver	October 28,	1986	1987:39
Sodium cyanide	February 7,	2001	2001:20
Sodium Fluoride	September 15,	2004	2005:17
Sodium hydroxide	August 24,	2000	2000:22
Stearates, metallic, some	September 15,	1993	1994:30
Stearates, non-metallic, some	November 17,	1993	1994:30
Strontium	January 26,	1994	1994:30
Styrene	February 29,	1980	1981:21
revised	October 31,	1989	1991:8
Sulfur dioxide	April 25,	1985	1985:32
Sulfur fluorides	March 28,	1990	1991:8
Synthetic inorganic fibers	March 4,	1981	1982:9
revised	December 1,	1987	1988:32
revised	December 3,	2003	2005:7
Synthetic organic and inorganic fibers	May 30,	1990	1991:8
Talc dust	June 12,	1991	1992:6
Terpenes, mono-	February 17,	1987	1987:39
Tetrabromoethane	May 30,	1990	1991:8
Tetrachloroethane	June 4,	1997	1997:25
Tetrachloroethylene	February 29,	1980	1981:21
1,1,1,2-Tetrafluoroethane	March 29,	1995	1995:19
Tetrahydrofuran	October 31,	1989	1991:8
Tetranitromethane	April 4,	1989	1989:32
Thioglycolic acid	June 1,	1994	1994:30
Thiourea	December 1,	1987	1988:32
revised	June 2,	1999	1999:26
Thiram	October 31,	1989	1991:8
Thiurams, some	October 31,	1989	1991:8
Tin and inorganic tin compounds	October 22,	2003	2005:7
Titanium dioxide	February 21,	1989	1989:32
Toluene	February 29,	1980	1981:21
revised	February 6,	2002	2002:19
Toluene-2,4-diamine	November 1,	2000	2001:20
Toluene-2,6-diamine	November 1,	2000	2001:20
Toluene-2,4-diisocyanate	April 8,	1981	1982:9
revised	May 30,	2001	2001:20

Toluene-2,6-diisocyanate	April 8,	1981	1982:9
revised	May 30,	2001	2001:20
1,1,1-Trifluoroethane	February 24,	1999	1999:26
Trichlorobenzene	September 16,	1993	1993:37
1,1,1-Trichloroethane	March 4,	1981	1982:9
Trichloroethylene	December 14,	1979	1981:21
Trichlorofluoromethane	June 2,	1982	1982:24
1,1,2-Trichloro-1,2,2-trifluoroethane	June 2,	1982	1982:24
Triethanolamine	August 25,	1982	1983:36
revised	October 23,	2002	2003:16
Triethylamine	December 5,	1984	1985:32
Trimellitic anhydride	September 12,	1989	1991:8
Trimethylolpropane	November 16,	1994	1995:19
Trinitrotoluene	April 17,	1991	1992:6
Vanadium	March 15,	1983	1983:36
Vinyl acetate	June 6,	1989	1989:32
Vinyl toluene	December 12,	1990	1992:6
White spirit	December 16,	1986	1987:39
revised	November 13,	2006	2008;42:6
Wood dust	June 17,	1981	1982:9
revised	June 25,	2000	2000:22
Xylene	February 29,	1980	1981:21
revised	September 14,	2005	2005:17
Zinc	April 21,	1982	1982:24
Zinc chromate	May 24,	2000	2000:22
Zinc dimethyl dithiocarbamate	September 12,	1989	1991:8
Ziram	September 12,	1989	1991:8

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