NR 2006:11

Scientific Basis for Swedish Occupational Standards XXVII

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ARBETE OCH HÄLSA | VETENSKAPLIG SKRIFTSERIE

ISBN 978-91-7045-812-5 ISSN 0346-7821

Arbetslivsinstitutet
National Institute for Working Life

Arbete och Hälsa

Arbete och Hälsa (Work and Health) is a scientific report series published by the National Institute for Working Life. The series presents research by the Institute's own researchers as well as by others, both within and outside of Sweden. The series publishes scientific original works, dissertations, criteria documents and literature surveys.

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ARBETE OCH HÄLSA

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National Institute for Working Life,
S-113 91 Stockholm, Sweden

ISBN 13: 978-91–7045–812–5
ISSN 0346–7821
http://www.arbetslivsinstitutet.se/
Printed at Elanders Gotab, Stockholm

Preface

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

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

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

This is the 27th volume that is published and it contains consensus reports approved by the Criteria Group during the period October 2005 through June 2006. These and previously published consensus reports are listed in the Appendix (p 58).

Johan Högberg
Chairman

Johan Montelius
Secretary

The Criteria Group has the following membership (as of June, 2006)

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[1] The English translation of the consensus report in Swedish on White spirit, published in Arbete och Hälsa 2006:9, will be published elsewhere.

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

[3] Drafted by Johan Montelius, Department of Work and Health, National Institute for Working Life, Sweden.

[4] Drafted by Johan Högberg, Institute of Environmental Medicine, Karolinska Institutet and National Institute for Working Life, Sweden.

[5] Drafted by Christer Edling, Professor in Occupational Medicine.

Consensus Report for Ammonia

October 24, 2005

This Report is based primarily on a criteria document compiled by the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (28).

Chemical and physical data

CAS No.:	7664-41-7
Formula:	NH_3
Molecular weight:	17.03
Melting point:	77.7 °C
Boiling point:	- 33.4 °C
Vapor pressure:	857 kPa (20 °C)
(28% aqueous solution:	59 kPa)
Solubility in water:	529 g/l (20 °C)
PK_a	9.15 (37 °C)
Conversion factors:	1 mg/m^3 = 1.4 ppm
	1 ppm = 0.7 mg/m^3 (25 °C)

Ammonia at room temperature is a colorless gas with a penetrating odor. The reported odor threshold is 5 to 6 ppm (28). Ammonia gas can be condensed by cooling under high pressure, and then stored and transported as liquid ammonia (anhydrous ammonia) (23). Ammonia dissolves quite readily in water and often occurs as an aqueous solution, usually about 28 to 30%. Solutions that are more concentrated than about 25-30% tend to release gaseous ammonia at normal temperatures. Ammonia in water yields ammonium hydroxide and the aqueous solution is basic (28, 29, 33).

In Swedish industry, ammonia is used mostly as an intermediate in various processes. It is also used in production of commercial fertilizer, as a pH regulator, as a flux, in cleaners, in surface treatments, as a refrigerant and in paints (28, 29). Some liquid ammonia (anhydrous ammonia) is also sold and used as commercial fertilizer (37). Exposure to ammonia can also occur around farm animals, and ammonia is part of the natural nitrogen cycle (28).

Uptake, biotransformation, excretion

Ammonia occurs naturally in the body. It is created and used in protein metabolism and is a normal part of all tissues (24, 28). Nearly all ammonia formed in the intestine (mostly from food) is absorbed. The content of ammonia in arterial blood from healthy persons is usually around 45 μmol/l, but with physical

work the muscles produce ammonia and the blood level can rise. Elevated blood levels of ammonia can also result from disturbances of liver and kidney function. Under normal physiological conditions >98% is in the form of ammonium ions (14, 16, 28, 29).

Occupational exposure to ammonia occurs primarily via inhalation (28). Ammonia is hygroscopic, and is usually absorbed in the upper respiratory passages. With high humidity and aerosol formation, however, uptake can occur further down in the lungs (28). Experiments with volunteers showed that with exposure to 56 – 500 ppm ammonia for up to 2 minutes, retention was about 92% and independent of the exposure level (25). With exposure to 500 ppm for 15 or 30 minutes, retention at equilibrium (after 10 to 27 minutes) was reported to be on average 23% (36). A calculation made by WHO indicates that exposure to 25 ppm ammonia would raise the blood level of ammonia by only 0.09 mg/l (5 μmol/l), assuming 30% retention and absorption. This level is about 10% above the fasting level in arterial blood (24, 42). Concentrations that damage the skin probably result in skin absorption, but there are no quantitative data (28).

Absorption and distribution of ammonia are highly dependent on pH. Non-ionized ammonia, which is more readily soluble in fat, diffuses freely in the cells, whereas the ammonium ion penetrates the cell membrane to a lesser extent. Ammonia is metabolized primarily in the liver, where it is rapidly transformed to urea with the aid of several different enzymes. The urea can be excreted in urine. The other major metabolism pathway in the liver leads to formation of glutamine via the enzyme glutamine synthetase. Glutamine can also be synthetized in other organs, and this is the primary detoxification mechanism for ammonia in tissues such as the brain. However, glutamine synthetase in the brain can not be induced with hyperammonemia; thus in such circumstances ammonia concentrations can rise considerably (16, 28). Glutamine is split into ammonia and glutamate by the enzyme glutaminase. In the kidneys this can lead to excretion as ammonium ions in urine, which is relevant to the acid-base balance in the body (28, 29). A little bit of ammonia is also eliminated in exhaled air, probably due to synthesis of ammonia from urea in saliva (28).

Toxic effects

Ammonia is irritating and caustic to skin and mucous membranes. The local effects are due mostly to the strong alkalinity of the substance. Since ammonia dissolves readily in water, it affects primarily the mucous membranes of eyes and upper respiratory passages, but at higher air concentrations the bronchi and lungs can also be affected. Ammonia can also cause sensory irritation of airways via the trigeminus nerve (6, 9, 28, 33, 39).

Human data

About 30 minutes of exposure to 2500 – 4500 ppm ammonia has been reported to be potentially lethal (3, 6, 28). The most common cause of death after acute

exposure to high concentrations of ammonia (gas or anhydrous liquid) is laryngeal or pulmonary edema (23). High, brief exposures cause immediate damage, with inflammation in respiratory passages (e.g. laryngitis, tracheobronchitis, pneumonia), and can also have chronic effects in the form of reduced lung function (15, 28). High, acute exposure to ammonia can cause irritant-induced asthma (reactive airways dysfunction syndrome, or RADS). In addition, exposure to ammonia can exacerbate pre-existing asthma (6, 7, 12). RADS was diagnosed in two painters exposed to ammonia and other substances for 12 hours while spray-painting an apartment. The workers used only paper masks for protection, and ventilation was extremely poor. Symptoms (general weakness, nausea, coughing, breathlessness, chest tightness, wheezing, paint taste in the mouth) began to show up after 12 hours of exposure. Both subjects had lower lung function and elevated bronchial reactivity to metacholine, and were hospitalized for two weeks (preliminary diagnosis acute chemical bronchitis). Four months after the incident the painters still had symptoms in the form of coughing, wheezing, breathlessness with exertion, and increased sensitivity to non-specific stimuli such as cold or smoke (11).

In an older study, 7 volunteers were exposed via breathing masks (covering nose and mouth) to 500 ppm anhydrous ammonia for 30 minutes. Reduced sensitivity in the skin covered by the mask and irritation of nose and throat, but no coughing, was reported. Only two people managed to breathe through their noses during the entire exposure. In previous experiments with higher concentrations (1000 ppm) the exposure was reported to result in immediate coughing (36).

Sixteen subjects were exposed to 50, 80, 110 or 140 ppm ammonia for 2 hours: no noteworthy effects were reported on vital capacity, FEV_1 or FIV_1 (reductions ≤10%) at any exposure level. There were concentration-dependent increases in subjective estimates of eye irritation, nasal irritation and throat irritation, as well as coughing, at 50 – 110 ppm, at 50 ppm generally regarded as slight ("just perceptible" to "distinctly perceptible"). Eight of the subjects described 140 ppm as strongly irritating and intolerable for 2 hours (43). In another study, 10 minutes of ammonia exposure was reported to be moderately irritating by 4 of 6 subjects at 50 ppm, and at 30 ppm irritation of eyes and nose was reported to be none or barely noticeable (30). In one report (Keplinger *et al*. 1973, cited in Reference 39), nasal dryness was reported with 5 minutes at exposure to 32 ppm (1 subject) and 50 ppm (2 subjects), and irritation of eyes, nose and throat was reported at 72 ppm. The concentrations are approximate (not measured directly). A recently published study reports no significant changes in lung function (FEV_1, diffusion capacity) or bronchial hyperreactivity (metacholine provocation) when 6 healthy persons and 8 persons with mild asthma were exposed to 16 – 25 ppm ammonia for 30 minutes (35).

In a German study (21), 43 persons (10 of them regularly exposed to ammonia at work) were exposed for five days to increasing concentrations of ammonia. They were exposed for 4 hours/day to 0 ppm on day one, 10 ppm on day 2, 20 ppm on day 3, 20 ppm plus 40 ppm for 2 x 30 minutes on day 4, and 50 ppm on day 5. No significant increase of inflammatory markers was found in nasal lavage

and there were no significant changes in measurement of nasal airway resistance, tear flow, bronchial reactivity or lung function. No effects on cognitive function were observed. However, increasing discomfort with increasing ammonia concentration was seen when assessing acute and irritative effects together (SPES questionnaire), intensity of irritation (eyes, nose), and respiratory symptoms (chest tightness, coughing, breathlessness). In the subjects accustomed to exposure, a significant increase of irritation symptoms was reported only at 50 ppm and there was no significant increase of respiratory symptoms at any exposure level. In persons unaccustomed to exposure, both irritation symptoms and respiratory symptoms increased significantly with increasing exposure, but the exposure levels at which the increases became significant (besides 50 ppm) is not clear. The rankings of irritation symptoms and of acute and irritative discomfort together were indicative of slight to extremely slight discomfort at 10 – 20 ppm. The rankings at 50 ppm showed very slight discomfort as a group average, although some individuals reported more noticeable discomfort at this concentration. Somewhat reddened conjunctiva were observed at 50 ppm in 3 of the 33 subjects unaccustomed to exposure. This group described the odor as unpleasant at 10 ppm and as fairly strong to strong at 50 ppm (21).

In a Swedish study, 12 healthy subjects were exposed to 0, 5 or 25 ppm ammonia in an exposure chamber for 3 hours (38). The exposure yielded no indications of inflammation in upper respiratory passages (nasal lavage), effects on lung function or increased bronchial reactivity to metacholine, although dose-dependent increases in subjective estimates of various symptoms were seen on a rating scale (VAS, Visual Analogue Scale). The increases were significant for eye irritation ($p<0.01$), dizziness ($p<0.05$), and feeling of intoxication ($p<0.05$) after the 5 ppm exposure, although the assessments made during the exposure were low (for eye irritation “hardly at all” on the scale). At 25 ppm there were significant increases in estimates of all listed irritation and CNS-related symptoms, and no indications of adaptation were seen. Average estimates during the exposure were still quite low, however: irritation in eyes, nose, and throat/airways, breathing difficulty and nausea got verbal rankings in the area “somewhat” on the VAS scale. For dizziness, headache and feeling of intoxication the estimates were even lower (38).

In a poorly reported study (17), 6 subjects were exposed on different schedules to 25, 50 or 100 ppm ammonia for 2 to 6 hours/day, 5 days/week for up to 6 weeks. Mild irritation of eyes, nose and throat was reportedly observed in subsequent medical examinations, but tolerance development was suggested and the subjects experienced no discomfort after the first week. No clear dose-effect correlation was seen (17).

In a study of 58 workers exposed to ammonia in the production of sodium carbonate, tests of lung function showed no differences from controls. Nor was there any observed difference in prevalence of symptoms involving respiratory passages, eyes or skin, although the exposed workers reported that some symptoms (including coughing, eye irritation) were more severe with exposure. There was no discernible difference between the groups in tests of odor threshold

during the workweek. Average ammonia exposure for the entire group was 9.2 ppm (time-weighted average, 8.4 hours). Exposure levels were reported to be below 50 ppm and in most cases below 25 ppm (22).

In a study of 161 workers in two fertilizer factories and 355 unexposed persons, a questionnaire indicated significantly higher relative risks of respiratory symptoms (coughing, mucus, wheezing, breathlessness) in factory A, but not in factory B. In factory A the air concentration of ammonia (8-hour) was 2 – 130.4 mg/m^3 (2.8 – 183 ppm) and in factory B 0.02 – 7 mg/m^3 (0.03 – 9.8 ppm). In factory A the geometric means were below 18 mg/m^3 (25 ppm) except in the packing area (18.6 mg/m^3) and the storage area for urea (115.1 mg/m^3) (stationary samplers, 8-hour samples). The urea storage area was not to be entered without "full protective clothing". According to the authors there were no other substances at the workplace besides ammonia that could affect respiratory passages. The production processes had not been changed since production began, and the measured ammonia levels were therefore considered representative. The exposed workers had been employed for an average of 51.8 months. When they were divided into exposure groups, significantly higher relative risks for coughing, mucus, wheezing, breathlessness and diagnosed asthma were seen for those exposed to average ammonia levels above 25 ppm, but for wheezing alone at average levels at or below 25 ppm. A calculation based on cumulative ammonia concentration yielded significant increases of respiratory symptoms as well as asthma and chronic bronchitis at levels >50 mg/m^3-year (>70 ppm-year), but for wheezing alone at levels ≤50 mg/m^3-year (≤70 ppm-year). It is also reported that most of the asthma cases worked in locations with "high" ammonia concentrations (5).

In a later study, the same authors report data on lung function for 73 exposed workers and 348 controls (probably from the above population). Somewhat lower lung function (FEV_1 and FVC in % of expected values) was noted in highly exposed workers when compared to a group with lower exposure, but not when compared to the unexposed group. FEV_1 in % of expected value, and FEV_1/FVC in % of expected value, were significantly lower for exposed workers with symptoms than for those without symptoms. FEV_1 in % of expected value was also significantly lower for the group of exposed non-smokers with symptoms. The ammonia concentrations (4-hour samples) ranged from 2 to 130.4 mg/m^3 (77 exposed workers). The geometric means were 5.5 mg/m^3 (range 2 – 8.1 mg/m^3) and 5.0 mg/m^3 (range 2.6 – 15 mg/m^3) in two departments, 18.6 mg/m^3 (range 10 – 27.1 mg/m^3) in the packing area and 115.1 mg/m^3 (range 90 – 130.4 mg/m^3) in the urea storage area (2).

Coughing, breathlessness and wheezing were reported in a person who had been using a silver polish containing ammonia in a small, poorly ventilated room. He had no previous history of asthma, and began to develop symptoms after 5 months of employment. He reported a strong odor of ammonia during his work, and measurements in the breathing zone showed 8 – 15 ppm. In addition to ammonia, the polish contained isopropyl alcohol, clay, fatty acid and water. He had no symptoms when he used a brass polish that produced an air concentration

of <1 ppm ammonia (27). In a controlled exposure, he developed rhinitis, watery eyes, and coughing after about 15 minutes of using the symptom-producing polish. Rhonchus was noted in both lungs. PEFR dropped by 42%, rose again after treatment with medicine to reduce asthma symptoms, and six hours later again dropped by 18%. In another controlled exposure, he was exposed to 12 ppm ammonia and within two minutes had an asthma attack with rhonchus in both lungs; PEFR fell by about 55%. Histamine provocation showed non-specific bronchial hyperreactivity (27). No delayed reaction was reported after the exposure to 12 ppm ammonia. A causal connection between exposure to low concentrations of ammonia and induction of asthma can not be established on the basis of this study.

Correlations between exposure to air pollutants in barns, stables and henhouses and increased occurrence of respiratory symptoms, bronchial inflammation and reduced lung function have been reported in some studies. The extent to which ammonia contributed to these effects is not clear, however, since the workers were also exposed to other substances including organic dust and endotoxins (26, 28, 34).

There are numerous reported cases of severe eye damage, including glaucoma and cataracts, attributed to a spray or splash of ammonia either in anhydrous form or as a concentrated solution. When one drop of 9% ammonium hydroxide solution was inadvertently dropped into one eye, most of the corneal epithelium was destroyed despite flushing the eye with water within 10 seconds. The eye healed in 3 to 4 days with no lasting damage (20). Severe skin damage has also been reported, especially in connection with using anhydrous ammonia as fertilizer in agriculture (3, 45).

Animal data

The LC_{50} for laboratory rodents is reported to be about 10,000 to 40,000 ppm for 10 minutes of exposure and 4,230 – 16,600 ppm for 1 hour of exposure (28). No acute effects (hypo- or hyperactivity, spasms) were observed in rats exposed for 2 hours to 121 ppm (Alpatov & Mikhailov 1963, cited in Reference 24). The RD_{50} (the concentration that produces a 50% reduction in respiratory rate), a measure of respiratory irritation, has been reported to be about 260 – 300 ppm for mice (15 – 30 minutes) (8, 28, 46).

No indications of toxicity were reported in a study (13) in which rats, rabbits, guinea pigs, dogs and monkeys were exposed to 56 ppm for 114 days, and rats were exposed to 178 ppm for 90 days. No noteworthy changes were observed, either in histopathological examinations (including lungs, liver, kidneys, heart, spleen) or in various biochemical and hematological parameters. In a sketchily described study, no toxic effects were observed in rats after two months of exposure to 57 ppm; however, histological changes (indications of inflammation) were observed in lungs, but not in other organs, at 143 ppm (Alpatov & Mikhailov 1963, cited in Reference 24). In some other rat studies as well, constant exposure to 150 – 200 ppm for a few weeks up to a few months has been reported to result

in histopathological changes in airways (e.g. loss of cilia, hyperplasia) (10, 19). In an older study in which guinea pigs were exposed to about 170 ppm (140 – 200 ppm) ammonia 6 hours/day, 5 days/week for up to 18 weeks, no significant changes were observed in microscopic examinations of animals killed after 6 and 12 weeks (44). The animals that were killed after 18 weeks, however, had slight changes in spleen, kidneys, adrenals and liver. The most pronounced changes were in the spleen (including congestion, hemosiderin). An incompletely described experiment, in which mice were exposed to vapor from a 12% ammonia solution 15 minutes/day, 6 days/week for 4 to 8 weeks, reports effects on enzymes (succinate dehydrogenase, acidic and alkalic phosphatases, non-specific esterases) and histological changes in respiratory passages (loss of cilia, epithelial hyperplasia, squamous cell metaplasia, dysplasia in nasal epithelium etc.) that became more pronounced with increasing length of exposure (18).

Rats were exposed to 25 – 250 ppm ammonia for a week and then given nasal inoculations of *Mycoplasma pulmonis*, after which the exposures were continued for a further 4 to 6 weeks. Indications of more severe mycoplasma infections were seen at all concentrations. The prevalence of pneumonitis also showed a tendency to increase with concentration (10). In another study, cell-mediated immune response to provocation with a tuberculin derivative was reduced in guinea pigs that were exposed to 90 ppm ammonia for 3 weeks (40).

Genotoxicity

There are few studies. Mutagenic effects have been reported in a few studies at toxic levels of ammonia (gas, ion form), but no conclusions can be drawn from these data (28).

Carcinogenicity

Mice were exposed to vapor from a 12% ammonia solution 15 minutes/day, 6 days/week: histological changes, increasing with exposure, were observed in airways. Ten exposed animals and 5 controls were killed each week in weeks 4 – 8. In week 6, epithelial hyperplasia was seen and 4 animals had flecks of squamous cell metaplasia. In week 7, 3 animals had dysplasia in nasal epithelium and one animal had carcinoma in one nostril. Changes observed in week 8 included adenocarcinoma in the nasal mucosa of one animal (18). The study is not fully reported; there are no weight curves or other information on effects on the mice. The latency time is remarkably short.

No carcinogenic effects were observed in mice after lifelong administration of 0.1, 0.2 or 0.3% ammonium hydroxide in drinking water (41). Other studies with oral administration of ammonia suggest that the ammonium ion can contribute to cancer development by functioning as a promoter (28).

Effects on reproduction

No toxicity studies of effects on human reproduction and no inhalation studies of effects on animal reproduction were found (28). Rats exposed to ammonia prenatally and in breast milk via oral administration of high doses of ammonium acetate to their dams (20% w/w in feed, equivalent to about 4 g ammonium ion/kg b.w./day) showed inhibited growth, poor function of N-methyl-D-aspartate receptors in the CNS and effects on learning (1, 32). No mention is made of toxic effects on the dams, but toxic effects can be expected since growth inhibition was seen in adult male rats exposed in the same way in another study (4). In the study by Aguilar *et al*. (1) that showed effects on learning, the rats were exposed (via feed) after weaning also, and there was no adequate control group. For these reasons no conclusions can be drawn from these studies regarding toxic effects of ammonia on reproduction.

Dose-effect / dose-response relationships

A significant increase of eye irritation ($p<0.01$) was reported by subjects in a study in which they were exposed to 5 ppm for 3 hours, although the assessments they made during the exposure were low: equivalent to "hardly at all" (38). In this study, therefore, the NOAEL was concluded to be 5 ppm. At 25 ppm the assessments of all the discomfort and CNS effects on the questionnaire were significantly higher, and no indication of adaptation was observed. The average estimate during the exposure for symptoms of irritation in eyes, nose and throat/respiratory passages, breathing difficulty and nausea was in the neighborhood of "somewhat". For dizziness, headache and feelings of intoxication, the estimates were lower (38). In another study with short-term exposure to 10 – 50 ppm, subjects reported increasing discomfort with increasing ammonia concentration, for acute and irritative discomfort together, for symptoms of irritation in eyes and nose and for respiratory symptoms (chest tightness, coughing, breathlessness). At 50 ppm, persons accustomed to exposure reported significantly more pronounced irritation symptoms in eyes and nose, compared to zero exposure, but there was no significant increase of respiratory symptoms at any exposure level. Persons unaccustomed to exposure are more sensitive, but it is not clear at what exposure levels the increases of irritation and respiratory symptoms became significant. No indications of inflammation in upper airways, effects on lung function or increased bronchial reactivity were reported in either of these studies (21, 38). Some subjects reported that 140 ppm was extremely irritating and intolerable for 2 hours (43).

Few reliable measures of occupational exposure to ammonia have been reported. There is also the problem of mixed exposures, which makes it difficult to sort out the effects of ammonia alone. In a study of workers exposed to air concentrations of ammonia averaging 9.2 ppm around production of sodium carbonate, lung function, prevalence of reported eye, nose or respiratory symptoms, and sense of smell were no different from controls. The exposed

persons reported that some symptoms (including coughing and eye irritation) were worse with exposure (22). Nor was there any significant increase in relative risk for respiratory symptoms (coughing, mucus, wheezing, breathlessness) in the workers in another study, who were exposed to air concentrations (8-hour measurements) of 0.03 – 9.8 ppm (5). A significantly higher relative risk of wheezing was reported at an average ammonia level of ≤25 ppm, and for respiratory symptoms and asthma at average levels of >25 ppm (5). In calculating the cumulative ammonia concentration, a significant increase of respiratory symptoms, as well as of asthma and chronic bronchitis, was noted at levels >50 mg/m^3-year (>70 ppm-year), but only wheezing at levels ≤50 mg/m^3-year (≤70 ppm-year). Smoking may have influenced the results, but the ammonia concentration was the only significant variable for asthma and wheezing/breathlessness (5).

Exacerbation of asthma that is not caused primarily by factors in the work environment, as well as the appearance of asthma, have been reported in several studies with high, acute exposure to ammonia (6, 7, 11, 12). However, no significant changes in results of lung function tests and tests of bronchial hyperreactivity were reported when persons with mild asthma were exposed to 16 – 25 ppm ammonia for 30 minutes (35). In a controlled study with exposure to 12 ppm ammonia, ammonia was identified as the etiological agent for asthma in a person who had been occupationally exposed to 8 – 15 ppm ammonia for 5 months while using silver polish (27). No other reports have been found of asthma with exposure to ammonia alone at such low air concentrations, and on the basis of present knowledge it is impossible to say whether low exposure to ammonia without previous high exposure can cause asthma.

Dose-effect relationships in people exposed to ammonia by inhalation are summarized in Table 1.

Dose-effect relationships observed in inhalation experiments with animals are summarized in Table 2.

Conclusions

The critical effect of exposure to ammonia is irritation of eyes and respiratory passages. Slight symptoms of irritation have been reported by experimentally exposed persons with short-term exposure to air concentrations around 20 – 25 ppm. Some eye discomfort has been reported at lower concentrations. One study of ammonia-exposed workers suggests that wheezing can appear at air concentrations below 25 ppm.

High, acute exposures can cause laryngeal and pulmonary edema, sometimes with fatal outcome. Appearance of asthma symptoms in direct connection to exposure to high concentrations of ammonia has also been reported. Ammonia can also intensify asthma caused by factors outside the work environment. Ammonia in anhydrous form and concentrated ammonia solutions can cause severe burns if they come into direct contact with skin or mucous membranes.

Table 1. Dose-effect relationships observed in humans exposed to ammonia by inhalation.

Concentration		Duration	Number exposed	Effects	Ref.
mg/m^3	ppm				
3.5	5	180 min	12	No indication of inflammation in upper airways, no increase in bronchial reactivity, no effect on lung function. Significantly higher subjective estimates of eye irritation ($p<0.01$), dizziness ($p<0.05$) and feeling of intoxication ($p<0.05$), although estimates during the exposure were low. Subjective estimate of eye irritation was "hardly at all".	38
6.4	9.2[1]	occupational exposure	58	No differences from controls in lung function (FVC, FEV_1, FEV_1/FVC, FEF_{50}, FEF_{75}) or prevalence of reported symptoms involving respiratory system, eyes and skin. No effect on sense of smell during the work week.	22
7	10	240 min	43	No indications of inflammation in upper airways, no increase in bronchial reactivity, no effects on lung function. Unaccustomed persons reported increased irritation (eyes, nose) and respiratory symptoms, but the significance is unclear. Some discomfort from the odor.	21
11-18	16-25	30 min	6 healthy subjects + 8 with mild asthma	No significant effect on FEV_1, diffusion capacity in lungs or bronchial hyperreactivity with metacholine provocation in either group.	35
14	20	240 min	43	No indication of inflammation in upper respiratory passages, no increase in bronchial reactivity, no effects on lung function. Unaccustomed persons: higher estimates of irritation (eyes, nose) and respiratory symptoms, but significance unclear. Odor unpleasant.	21
≤18	≤25[2]	occupational exposure	138	Higher relative risk of wheezing (RR 2.26; 95% CI: 1.32 – 3.88).	5
>18	>25[3]	occupational exposure	17	Higher relative risks for: coughing (RR 3.48; 95% CI 1.84 – 6.57) mucus (RR 3.75; 95% CI 1.97 – 7.11) wheezing (RR 5.01; 95% CI 2.38 – 10.57) breathlessness (RR 4.57; 95% CI 2.37 – 8.81) asthma (RR 4.32; 95% CI 2.08 – 8.98)	5

Table 1. Cont.

Concentration mg/m^3	Concentration ppm	Duration	Number exposed	Effects	Ref.
17.5	25	180 min	12	No indication of inflammation in upper respiratory passages, no increase in bronchial reactivity, no effects on lung function. Significantly higher estimates of irritation and CNS-related symptoms. Estimates during exposure were "somewhat" for irritation of eyes, nose and throat/airways; breathing difficulty and nausea, and even lower for dizziness, headache and feeling of intoxication.	38
21	30	10 min	5	No irritation (3/5) or barely noticeable irritation (2/5) of eyes and nose.	30
14 + 28	20 + 40	240 min + 60 min	43	No indications of inflammation in upper airways, no increase in bronchial reactivity, no effects on lung function. Unaccustomed persons: higher estimates of irritation (eyes, nose) and respiratory symptoms; significance unclear. Odor unpleasant.	21
35	50	240 min	43	No indications of inflammation in upper airways, no increase in bronchial reactivity, no effects on lung function. Unaccustomed persons: significantly higher estimates of irritation (eyes, nose) and respiratory symptoms; swelling/redness of conjunctiva in 3/33. Odor unpleasant. Accustomed persons: significantly higher estimates of irritation (eyes, nose). Some discomfort from odor.	21
35	50	10 min	6	Moderate irritation of eyes and nose in 4/6: barely noticeable irritation in 1/6.	30
35	50	120 min	16	VC, FEV_1 and FIV_1 reduced by no more than 10%. Slight/relatively slight irritation of eyes, nose and throat.	43
70	100	5-30 seconds	23	Duration-dependent increase of airway resistance in nose, nasal irritation in 11/23.	31
77	110	120 min	16	VC, FEV_1 and FIV_1 reduced by ≤10%. Irritation of eyes, nose, throat; coughing.	43
98	140	up to 120 min.	16	VC, FEV_1 and FIV_1 reduced ≤10%. Intolerable for 8/16.	42

[1] Time-weighted average (TWA); personal monitors, average sampling time 8.4 hours (exposure levels <50 ppm, in most cases <25 ppm).
[2] Geometric mean; stationary monitors, 8-hour shift.
[3] Geometric mean; stationary monitors, 8-hour shift (maximum exposure level 182 ppm)

Table 2. Dose-effect relationships observed in animals experimentally exposed to ammonia by inhalation.

Exposure (ppm)	Exposure time	Species	Effects	Ref.
25	7 days + 30-42 days constant	rat	More severe mycoplasma infections after nasal inoculation with *Mycoplasma pulmonis*.	10
56	114 days constant	rat rabbit guinea pig dog monkey	No indications of toxicity, no noteworthy histopathological changes.	13
57	2 months	rat	No toxic effects.	Alpatov & Mikhailov 1963, cited in Ref. 24
90	3 weeks constant	guinea pig	Reduced cell-mediated immune response	40
121	2 hours	rat	No acute effects (hypo- or hyperactivity, spasms).	Alpatov & Mikhailov 1963, cited in Ref. 24
143	2 months	rat	Histological changes in lungs (including small areas of interstitial pneumonia), no changes in other examined organs.	Alpatov & Mikhailov 1963, cited in Ref. 24
150	75 days constant	rat	Histological changes (including hyperplasia) in olfactory and respiratory epithelium in nasal cavity.	10
170	6 hours/day 5 days/week up to 18 weeks	guinea pig	After 18 weeks: relatively mild histological changes in spleen, kidneys, adrenals and liver.	44
178	90 days constant	rat	No indications of toxicity, no noteworthy histological or hematological changes, no histochemical changes in liver.	13
200	4 – 12 days constant	rat	Histopathological changes in trachea, including loss of cilia and hyperplasia.	19
257	15 min.	mouse	RD_{50}	46
303	30 min.	mouse	RD_{50}	8
367	90 days constant	rat	Slight irritation in 25% of animals.	13
658	90 days constant	rat rabbit guinea pig dog monkey	Clear eye irritation in dogs and rabbits, erosion of 1/4 - 1/2 of the cornea in rabbits. Histopathological changes in lungs, kidneys, heart and liver. 13/15 rats died, 4/15 guinea pigs died.	13

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Consensus Report for Penicillins

November 23, 2005

This Report is based primarily on a criteria document compiled by the Nordic Expert Group (37). Like the criteria document, it is limited to the effects of penicillins that are relevant in the context of occupational health, i.e. effects of therapeutic use are not taken up. The most recent literature search was made in April of 2005.

Chemical and physical data. Uses

Penicillins belong to the group of β-lactam antibiotics. Other antibiotics in this group include cephalosporins, carbapenems and monobactams (59).

Penicillins can be divided into naturally occurring penicillins, penicillinase-resistant penicillins, aminopenicillins, carboxypenicillins and ureidopenicillins (see Table 1). They all have the same basic structure: 6-aminopenicillanic acid (R_1 and R_2 = H in Figure 1), a linking of the amino acids L-cysteine and D-valine forming a cyclic amide (β-lactam), which is attached to an imidazol ring. The antibacterial properties of penicillins are attributed to their high affinity to enzymes that synthesize the bacterial cell wall and to the reactivity resulting from the flat arrangement of the ring system, with the high tension in the lactam ring. Many penicillins are inactivated by enzymes, e.g. β-lactamases, which are produced by some bacteria (27, 37, 65).

A large number of penicillins have been isolated and synthesized: differences in the structure of the side chains R_1 and R_2 (Figure 1) give them different antibiotic spectra, pharmacokinetics, and acid and β-lactamase stability. Examples of penicillin structure and characteristics are shown in Table 1 (27, 65). As a rule, penicillin salts of potassium, sodium and calcium are easily soluble in water, whereas other counterions such as procaine and benzathine produce salts that dissolve less readily (27).

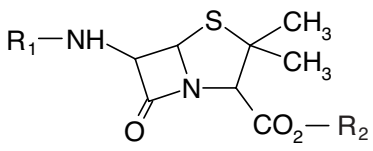
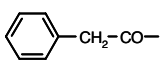
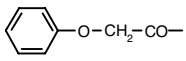
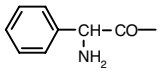
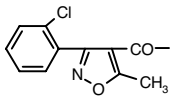
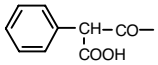
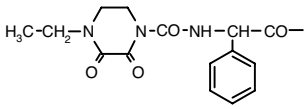


Figure 1. A generalized diagram of the basic structure of penicillins.

Table 1. Examples of side-chain structure R_1 (see Figure 1) and characteristics of some penicillins (27, 65).

Name	Synonym	Structure[1], R_1	Characteristics[2]
Penicillin G[3]	benzylpenicillin		G(+) sensitive to acid and to β-lactamase
Penicillin V[3]	phenoxymethyl penicillin		G(+) acid-stable, sensitive to β-lactamase
Ampicillin[4]	D-α-(-)-aminobenzyl penicillin		G(+) and G(–) acid-stable, sensitive to β-lactamase
Cloxacillin[5]	3-o-chlorophenyl-5-methyl-4-isoxazolyl penicillin		G(+) acid-stable β-lactamase resistant
Carbenicillin[6]	α-carboxybenzyl penicillin		G(+) and G(–) sensitive to acid and to β-lactamase
Piperacillin[7]	4-ethyl-2,3-dioxopiperazine-carbonyl ampicillin		G(+) and G(–) sensitive to acid and to β-lactamase

[1] in all these penicillins R_2 = H (see Figure 1), pKa = 2.6 – 2.8
[2] G(+) = effective primarily against gram-positive bacteria; G(-) = effective primarily against gram-negative bacteria
[3] naturally occurring penicillin
[4] aminopenicillin
[5] penicillinase-resistant penicillin
[6] carboxypenicillin
[7] ureidopenicillin/piperazine penicillin

Penicillins are solid powders with very low vapor pressure. Exposure to airborne penicillin is therefore usually due to aerosols from powders or solutions containing penicillin (37).

A method for qualitative and quantitative determination of penicillin in workplace air has been described (19). Qualitative (semi-quantitative) determination of penicillin is based on the number of inhibition zones in a Petri dish containing penicillin-sensitive bacteria in an agar gel that has been placed out in the workplace. For quantitative determination of penicillin in air, air samples are taken on a filter with the aid of an air pump. The amount of penicillin on the filter is then determined by extraction and a bioassay that measures the inhibition of penicillin-sensitive bacteria caused by the extract (19).

Some studies report air concentrations of penicillins around penicillin production and handling of penicillin preparations (16, 23, 61), see below.

A NIOSH report (23) contains measures of total dust in a factory producing 17 different formulations from 4 penicillins. Total dust (measured by personal monitors) in the breathing zone of production workers (weighing, granulating, capsule filling, tablet pressing, powder filling) was 6.0 mg/m^3. It was 0.3 mg/m^3 for employees working with packaging and 0.5 mg/m^3 for quality controllers. It is not clear what proportion of the total dust was penicillin.

Shmunes *et al*. (61) reported ampicillin levels of 3.7 – 262 mg/m^3 around mixing, capsule filling and grinding and 0.005 – 0.789 mg/m^3 around packaging, and benzylpenicillin levels of 11 to 42,857 units/m^3 around charging of reactors (1 mg benzylpenicillin-potassium salt is equivalent to about 1500 units) in a factory making synthetic penicillin.

Air contents of amoxicillin (Imacillin®) were measured around preparation of an amoxicillin solution. Particle size in the aerosol was <3 μm and the average air concentration of amoxicillin was 1.2 μg/m^3 (range 0.69 – 2.95). Air concentrations were measured by collection on a filter for 5 minutes and subsequent extraction and analysis with traditional microbiological methods (16).

It is difficult to estimate the number of persons occupationally exposed to penicillin in Sweden (37). Exposure occurs, or can be suspected to occur, for several different types of workers, such as:

- People who work with production, processing and formulation of penicillins and penicillin preparations in the pharmaceutical industry
- Pharmacists who prepare penicillin formulations
- Health care workers who administer penicillin preparations and care for patients.
- Veterinarians, farmers and fish farmers who treat animals with penicillin.
- Laboratory workers who use penicillin in research or in standard analyses.
- Persons who handle waste containing penicillin

People take penicillin in the form of tablets, capsules, mixtures, drops, infusions and injections, and animals are given tablets, mixtures, injections and preparations for topical treatment of udder inflammations. In Sweden in 1999, 8.1 doses of penicillin for human use were sold per 1000 inhabitants per day (2), equivalent to about 27 tons per year if it is assumed that a one-day dose is about 1 gram. A large portion of the Swedish population is therefore exposed to penicillin therapeutically. In veterinary medicine, 13.2 tons of benzylpenicillin (penicillin G) and 0.86 tons of ampicillin/amoxicillin were used in Sweden in 1993 (5).

A summary of penicillin preparations sold in Sweden in 2000 is given in Table 2. Structures, synonyms etc. are found in the criteria document (37).

Table 2. Penicillin preparations registered for use in Sweden in 2000 (37).

Name	CAS number	Formula	Mol weight
Procaine benzylpenicillin	54-35-3	$C_{16}H_{18}N_2O_4S \cdot C_{13}H_{20}N_2O_2$	571
Ampicillin, sodium salt	69-52-3	$C_{16}H_{19}N_3O_4S \cdot Na$	372
Benzylpenicillin, sodium salt	69-57-8	$C_{16}H_{18}N_2O_4S \cdot Na$	357
Penicillin V, potassium salt	132-98-9	$C_{16}H_{18}N_2O_5S \cdot K$	389
Cloxacillin, sodium salt	642-78-4	$C_{19}H_{18}ClN_3O_5S \cdot Na$	459
Benethamine penicillin	751-84-8	$C_{16}H_{18}N_2O_4S \cdot C_{15}H_{17}N$	546
Penicillin G benzanthine	1538-09-6	$(C_{16}H_{18}N_2O_4S)_2 \cdot C_{16}H_{20}N_2$	909
Penicillin G diethylaminoethyl ester	3689-73-4	$C_{22}H_{31}N_3O_4S$	434
Procaine penicillin	6130-64-9	$C_{16}H_{18}N_2O_4S \cdot C_{13}H_{20}N_2O_2 . H_2O$	589
Cloxacillin, sodium monohydrate	7081-44-9	$C_{19}H_{17}ClN_3O_5S \cdot Na \cdot H_20$	476
Dicloxacillin, sodium monohydrate	13412-64-1	$C_{19}H_{16}Cl_2N_3O_5S \cdot Na \cdot H_2O$	510
Globacillin	17243-38-8	$C_{16}H_{17}N_5O_4S$	375
Pivampicillin hydroklorid	26309-95-5	$C_{22}H_{29}N_3O_6S \cdot HCl$	500
Pivamdinocillin	32886-97-8	$C_{21}H_{33}N_3O_5S$	440
Selexid	32887-01-7	$C_{15}H_{23}N_3O_3S$	325
Pivmecillinam hydrochloride	32887-03-9	$C_{21}H_{33}N_3O_5S \cdot HCl$	476
Ampicillin pivaloyl-oxymethyl ester	33817-20-8	$C_{22}H_{29}N_3O_6S$	464
Bacampicillin hydrochloride	37661-08-8	$C_{21}H_{27}N_3O_7S \cdot HCl$	502
Flucloxacillin	58486-36-5	$(C_{19}H_{16}ClFN_3O_5S)_2 \cdot Mg \cdot 8H_2O$	1 074
Amoxicillin trihydrate	61336-70-7	$C_{16}H_{19}N_3O_5S \cdot 3H_2O$	419

In the 1995-2001 period there were 24 occupational injury reports citing penicillin as a possible cause. During this same period there were 18 further reports in which antibiotics or medicines were given as a possible cause. (The Work Injury Information System [InformationsSystemet om Arbetsskador, ISA], personal communication from Börje Bengtsson, Swedish Work Environment Authority).

Uptake, distribution, metabolism, excretion

Since penicillin is skin-sensitizing it clearly can penetrate the skin, but no quantitative data were found.

Only one study describing lung uptake of penicillin was found. Rats were exposed for 5 minutes to an aerosol (mass median aerodynamic diameter 2.92±0.05 μm) consisting of benzylpenicillin (1 mM) dissolved in a phosphate

buffer, after which the lungs were examined at intervals for remaining benzylpenicillin. The half time for benzylpenicillin in the lungs was 20.5 minutes (6).

Absorption in the digestive tract of oral doses of penicillins designed for oral use ranges from 30 to 90% (37). Maximum serum level is usually reached 1 to 2 hours after administration. Food intake can both retard and reduce absorption. If penicillin is taken directly after a meal, for example, serum levels are 30 to 60% lower than if the same dose had been taken on an empty stomach. Some penicillins, such as the ureidopenicillins, are poorly absorbed in the digestive tract, and others, such as benzylpenicillin, are broken down by gastric acid (27, 37, 65).

All penicillins are distributed well to most body tissues. There are a few exceptions, most notably prostate, eyes and cerebrospinal fluid. Penicillin in blood is reversibly bound to serum proteins in proportions ranging from 15% for aminopenicillins to 97% for dicloxacillins. About 50% of benzylpenicillin is bound to plasma proteins. Only the unbound fraction is biologically active (37, 65).

Most penicillin is excreted unchanged in urine, but a small portion is metabolized. Up to ten percent of the metabolites form covalent bonds to lysine and cysteine remnants in serum proteins, membrane proteins and microbial proteins. Most (95%) of these bound metabolites are penicilloyl-protein conjugates called "major determinants" because they are formed in the largest quantity. The rest of the bound metabolites are referred to as "minor determinants". These are less well defined, but consist of metabolites from unmodified penicillin, penicilloate, penilloate and possibly other breakdown products. Both the major determinants and the minor determinants have been shown to be involved in life-threatening allergic reactions to penicillin, the latter group possibly more often with anaphylactic shock. The penicilloyl group bound to polylysine (penicilloyl polylysine, PPL) is used in tests for penicillin allergy (9, 37, 59, 65, 66).

Penicillin and penicillin metabolites are rapidly excreted via the kidneys (glomerular filtration and tubular secretion). The half time in serum is brief, about 30 minutes for benzylpenicillin and 60 minutes for aminopenicillins. A few penicillins, e.g. cloxacillins, nafcillin, oxacillin and ureidopenicillins, are also excreted to some extent (20 to 30%) in bile (37, 65).

Toxic effects

Hypersensitivity reactions

Numerous articles (1, 4, 7, 8, 10-14, 17, 18, 20, 21, 23-26, 29, 31, 38-48, 50-58, 60-63) have been published describing hypersensitivity reactions after occupational exposure to penicillins. Most of the subjects were employed in pharmaceutical production, health care or veterinary medicine. The hypersensitivity reactions are of Type IV (allergic contact eczema) and Type I (IgE-mediated allergy) according to the classification system of Coombs and Gells (49). A case of penicillin-induced alveolitis has also been described (13).

Type I reactions are characterized by one or more symptoms or diagnoses, including urticaria, allergic rhinitis, sneezing, itching, conjunctivitis, angioedema,

digestive disorders, breathlessness, wheezing, asthma and anaphylactic shock. However, it is not always possible to show that IgE antibodies are involved in penicillin-induced immediate hypersensitivity reactions with exposure via respiratory passages or skin, and a still poorly understood non-IgE mediated immunological mechanism has been proposed (for more information refer to the Criteria Document, Reference 37). Further, a non-immunological mechanism, such as irritation caused by dust, can cause some similar symptoms (37).

Two studies giving exposure levels are described below. A few of the more informative case reports are described in the text and presented in Table 3. More case reports are described in the Criteria Document (37).

A NIOSH report (not vetted) (23) describes a study of lung function and occurrence of asthma-like symptoms (questionnaire) in workers exposed to penicillin powder and granules in a pharmaceutical factory. Four different penicillins (not further specified) were handled. Total dust was measured with personal monitors (see above). The 36 penicillin-exposed subjects (26 women and 10 men) were divided into three exposure groups based on job description: high (n = 10; 5.97 mg/m^3, range 2.48 – 12.47), medium (n = 7; 0.50 mg/m^3, range 0.08 – 1.48) and low (n = 19; 0.29 mg/m^3, range 0.12 – 0.45). Attacks of breathlessness and wheezing were more prevalent in the penicillin-exposed subjects (15 of 36; 42%) than in controls (2 of 27; 7%) consisting of 27 employees (23 women and 4 men) in the same factory who were not exposed to penicillin and whose total dust exposure was 0.30 mg/m^3 (0.20 – 0.74). When only the women in the groups were compared, there were also significantly higher prevalences of chronic cough (13 of 26 = 50%; controls 2 of 23 =9%), wheezing (14 of 26 = 54%; controls 2 of 23 = 9%) and breathlessness (9 of 26 = 35%; controls 1 of 23 = 4%). No dose-response relationship between asthma-like symptoms and exposure to penicillin dust could be identified, but the authors point out that several persons in the low-exposure group had previously worked in high-exposure parts of the factory but had been transferred for health reasons. Lung function tests (FEV_1) given before and after a workshift showed no difference between the exposed groups or between these groups and controls. The authors could not definitely state that asthma due to penicillin exposure occurred in the factory, since no effect was shown by the lung function tests. However, some of the workers with symptoms used broncho-dilators during their workshifts, and spirometry measurements were taken 6 hours after the beginning of exposure – possibly too early to show a reduction in FEV_1 (23).

Employees (169 volunteers of a total of 319) in a factory producing synthetic penicillins were studied by Shmunes *et al.* (61) to ascertain whether there were any correlations between immunological reactions, allergy symptoms and penicillin levels in the factory. Air concentrations of ampicillin were measured with personal monitors. The samples were collected on a millipore filter and quantified by the bioassay method described in Garth *et al.* (19). The volunteers were divided into 4 exposure groups: group A (n = 62) was exposed to <0.1 mg/m^3, group B (n = 49) to 0.1 – 9.9 mg/m^3, group C (n = 42) to 10 – 263 mg/m^3 and group D (n = 16) was exposed periodically. The workers were interviewed,

and only symptoms that appeared or became more pronounced since they were hired were noted: 67 persons reported one or more symptoms meeting this criterion. The most common symptoms were local rashes, runny noses with sneezing, general itching and itchy eyes. A few also reported swollen eyes, face and lips, urticaria, wheezing (2 persons), chronic diarrhea, "black hairy tongue" and/or eczema. Symptoms were significantly more frequent in groups B and C than in group A. There was also a significant correlation between symptoms and the occurrence of penicillin-specific IgG and/or IgM antibodies. On the other hand, no correlation was seen between the symptoms and duration of employment, age, or most recent known therapeutic use of penicillin. Prick tests with PPL (penicilloyl polylysine) were negative, and intradermal tests were weakly positive for one person and yielded unclear results for a few others. One of 9 patch-tested persons with eczema had a positive reaction to the penicillins he worked with (61). The authors point out that the highest measured dust levels were several times the threshold limit for inert nuisance dust (15 mg/m^3) and that this alone may have triggered most of the reported symptoms (rash, runny nose with sneezing, generalized itching and itchy eyes) via a non-immunological mechanism (61).

Three cases of penicillin sensitization are described in an article by Reisman and Arbesman (46). Case 1 is a woman who for 5 years distributed penicillin tablets to patients in a mental hospital. For 8 weeks she had been suffering from nasal congestion, rhinitis, generalized itchiness, conjunctivitis, coughing and wheezing at work. The symptoms appeared only while she was at work, beginning after about 30 minutes and becoming more severe during her shift. The last time she had received parenteral penicillin was three years previously. Intradermal tests with benzylpenicillin and PPL were positive (for benzylpenicillin strongly so), and within 5 minutes she had a severe systemic reaction with generalized urticaria, rhinitis, conjunctivitis, coughing and breathlessness. Case 2 describes a nurse who developed generalized urticaria within 10 minutes of swallowing two penicillin tablets. She responded rapidly to treatment with adrenaline and antihistamine. She had previously been treated with penicillin on several occasions without developing symptoms. Several weeks after this first reaction she began to develop generalized urticaria every day at work. Her duties included distributing medicine for oral use, but not giving injections. Intradermal tests with benzylpenicillin and PPL were strongly positive. Case 3 describes a male farmer, an atopic with allergic rhinitis and asthma, who on three occasions developed generalized itching, a swollen finger and asthma shortly after having injected cows with penicillin. He had once developed severe urticaria on his face right after his wife, who had just taken a penicillin tablet, touched him with her hand. The man had previously been treated numerous times with penicillin and had no problems. He had also been treating his cows with penicillin for some time (not specified) since his most recent penicillin treatments, and had no problems. An intradermal test with PPL was strongly positive. The authors conclude that the woman in the first case had been sensitized by repeated inhalation of low concentrations of penicillin and the woman in the second case had been sensitized by

either parenteral or inhalation exposure to penicillin. The man in the third case was probably sensitized by skin contact and inhalation of penicillin when he gave the penicillin to his cows. Penicillin-specific IgE antibodies were found in all three patients (46).

Three men who were exposed to ampicillin and other penicillins by skin contact and inhalation during ampicillin production developed rhinitis and asthma symptoms after about 2 years on the job. None of them had a history of asthma, hay fever or allergy to medicines before the exposure. Provocation tests with inhalation of one or more of the tested penicillins yielded an asthmatic reaction of the late type (3 to 16 hours after exposure) with FEV_1 reduction of more than 15%, and eosinophilia in the blood within 24 hours. Oral provocation triggered a late asthma reaction in two of the men, one of whom also developed urticaria. Prick tests with penicillins were negative (12). The authors concluded that the men had developed asthma as a result of inhaling penicillin dust, but no information is given on possible therapeutic penicillin use during the presumed two-year latency period.

In an Italian dermatology clinic, 3,758 eczema patients were patch-tested during 1968 – 1977, and 4,472 in 1978 – 1983, with a series of pharmaceuticals including penicillin. The proportion of positive patch tests for penicillin dropped from 4.6% in the first period to 0.6% in the second (1).

Among patients with work-related contact eczema who were patch-tested in a Polish dermatology clinic, a maximum of positive patch tests for penicillin was seen in the 1981 – 1985 period (9.8%). The percentage of positive patch tests then began to drop, and in 1996 – 1998 was down to 0.7%. The drop closely followed the reduction in use of benzylpenicillin, from 21 million capsules annually in 1989 to 4 million in 1998 (58).

It was judged from a review of medical histories that 39 workers exposed regularly to bacampicillin in a Swedish pharmaceutical production plant had developed bacampicillin hypersensitivity in the 1990 – 1998 period. Sixteen of the cases reported symptoms indicating Type 1 hypersensitivity (rhinitis), 19 indicating contact allergy (eczema), and 4 indicating both Type 1 and contact allergy. Patch tests with bacampicillin yielded 11 positive responses in the first group, 16 in the second group, and 3 in the third group. Prick tests were positive for 5 of 8 tested in the first group and 1 of 2 tested in the third group. In a lymphocyte transformation test (LTT) with bacampicillin, 87% of the 39 had a positive response (8). No air concentrations were given in the article, but there are some unpublished data. In 1977 air concentrations (total dust) were 2 to 90 mg/m^3 measured around charging of benzylpenicillin, and 0.4 to 1.2 mg/m^3 around removing the bacampicillin for drying. The production process was changed to a closed system in several steps implemented between 1995 and 2002. In 2001 concentrations were 16 and 0.1 mg/m^3 respectively, and in 2003, 0.3 and 0.1 mg/m^3 respectively. No new cases of hypersensitivity to penicillin have been recorded at the company since the production process was redesigned (personal communication 2005, Marie Haag Grönlund, AstraZeneca).

Table 3. Summary of some case reports of hypersensitivity reactions caused by penicillins.

Exposure situation or profession	Number of cases	Effects/test results	Ref.
Synthesis of pivmecillinam and pivampicillin	14	Frequently recurring symptoms such as rhinitis, eczema and urticaria in 6 employees working with penicillin synthesis, and rhinitis and conjunctivitis in 8 employees who packaged penicillin powder containing flavor additives. Three had symptoms of asthma. Latency time between start of exposure and appearance of symptoms varied considerably, from 1 week up to 5 years. Symptoms became more severe when exposure increased. Basophil histamine release tests were positive for 5 of the 14, and patch tests with various penicillins were positive for 4 of 9 tested.	38
Production of pivmecillinam and pivampicillin	45	In a factory producing penicillins, medical examinations and patch tests were given to 45 employees with eczema, especially on hands, arms, calves and face. All of them had positive results for at least one of the tested penicillins, and 29 for two or more of them; 5 reported symptoms of asthma (mostly of the late type), 17 reported hay fever symptoms, and 3 reported both types. Latency time between start of exposure and appearance of symptoms ranged from 1 week to 1 year. The factory was highly contaminated with penicillin dust. The total number of employees at the factory is not reported.	39
Penicillin production	1	One case of penicillin-induced allergic alveolitis with airways hyperreactiviity has been described: a 63-year-old woman who had been exposed to penicillin in a pharmaceutical factory for 12 years. After 5 years of employment she developed (after an hour or two at work) daily problems with coughing, breathlessness, wheezing, chest tightness and itching rash, sometimes accompanied by conjunctivitis and rhinitis. The symptoms became more severe with time. She was examined 18 months after she quit her job. An intradermal test with PPL yielded a reaction after 6 hours. A serum precipitin test was negative. An inhalation provocation test with benzylpenicillin (10 mg/m^3 for 60 minutes) gave no immediate reaction, but after 2 hours coughing, chest tightness and wheezing; FEV_1 dropped by 12% and FVC by 20%. D_{CO} dropped by 20% after 6 hours. There was no reaction to provocation with lactose alone. A transbronchial biopsy showed slight fibrosis. A metacholine test given 24 hours after the provocation test showed hyperreactive airways. A metacholine test given a little over a year later was normal.	13
Nurse	1	A nurse developed facial erythema with severe swelling and itching a few hours after she accidentally sprayed her face with a solution of benzylpenicillin while she was preparing an injection. A patch test with benzylpenicillin was positive, a prick test negative.	45
Nurses	21	In a patch-test study of 333 nurses, 21 had positive reactions to one or more of the tested penicillins. Although it is not stated clearly in the article, the 333 nurses probably had eczema.	56

Table 3. Cont.

Exposure situation or profession	Number of cases	Effects/test results	Ref.
Nurses	7	The study describes 6 nurses who had reactions after being in a room where penicillin injections were given. Four had anaphylactic shock, and one of these also had urticaria. One of the other two developed urticaria and rhinitis, the other urticaria only. A seventh nurse is also described, whose face became swollen after she inadvertently sprayed penicillin on it while preparing an injection. Two days later her hands began to itch, the urticaria spread, and her blood pressure fell drastically 10 minutes after she had prepared a penicillin solution.	53
Veterinarians	6	Six veterinarians with eczema, primarily on the hands, arms and face, had positive patch tests for penetamate (penicillin used on cows for local treatment of udder inflammation); 3 were also positive for benzylpenicillin.	24
Veterinarians	23	Of 37 veterinarians with debilitating eczema, 23 had positive patch tests for pentamate and 5 for benzylpenicillin.	26
Veterinarians	5	Of 34 veterinarians who had or had had eczema, 9 were judged to have occupation-related positive patch test reactions. Five of the 9 were positive for penetamate; 4 of these were also positive for other penicillins.	14
Nurse	1	A nurse developed eczema on her hands and in skin creases after about 5 years of work at a hospital. She also developed urticaria and shortness of breath after skin contact with a mezlocillin solution. Increasing discomfort caused her to change jobs 4 years later. An open patch test with mezlocillin produced a local urticarial reaction after 10 minutes. After 2 and 3 days an eczematous reaction was observed. Benzyl- and phenoxymethyl penicillin-specific IgE antibodies were identified. According to the authors, she was probably sensitized by occupational exposure.	29
Nurse	1	A nurse, after 15 years in the job, opened a package of amoxicillin and rapidly developed facial edema, rhinoconjunctivitis, breathlessness, and difficulty in speaking and swallowing. Prick tests were positive for amoxicillin and ampicillin. IgE antibodies were identified for benzyl- and phenoxymethyl penicillin, but not for amoxicillin or ampicillin. A histamine liberation test was positive for amoxicillin but not for ampicillin.	10
Nurses, veterinarians	11	Of 14,689 patients who were examined for suspected contact allergy at a Belgian dermatology clinic in the 1978 – 2001 period, occupational allergic contact eczema was diagnosed in 33 persons who worked in health care: 11 of them (7 nurses, 4 veterinarians) had positive patch tests for one or more penicillins.	20
Livestock breeder	1	A livestock breeder developed eczema on hands, face and both sides of the neck. His eczema became more severe every time he prepared feed, which involved mixing the feed with various antibiotics. Patch tests were positive for penicillin and some other antibiotics.	21

Experimental studies

No published animal studies on effects of penicillin inhalation were found in the literature searches (37).

The contact allergenic potential of benzylpenicillin has been studied with the "human maximization test" (32). Benzylpenicillin was classified on different occasions as a moderate or strong contact allergen (Grade III allergen; 32 – 52% sensitized, or Grade IV allergen, 56 – 80% sensitized) for humans (32). When benzylpenicillin was tested further in a modified test, the "reduced maximization test", it was found that repeated exposure to a concentration as low as 0.1% in vaseline could cause sensitization (33).

The contact allergenic potential of penicillins has been quantified in the Guinea Pig Maximization Test (GPMT). In four studies (3, 22, 34, 35) benzylpenicillin was classed as a Grade V allergen (81 – 100% sensitized animals) on the Magnusson-Kliegman scale (35), and in a fifth study (36) as a Grade IV contact allergen (65 – 80% sensitized animals). Bacampicillin and cloxicillin were also classified as Grade V allergens in these tests (34). There was a pronounced cross-reactivity between benzylpenicillin and bacampicillin, whereas the cross-reactivity between cloxacillin and the two others was more moderate (34).
For further details see (37).

Benzylpenicillin has also been tested in mice with the Local Lymph Node Assay (LLNA), and was found to be a potential skin sensitizer (3, 30). The results with mice indicated a weaker allergenic potential for benzylpenicillin than indicated by the above-described tests with humans and guinea pigs (37).

Other effects

A Russian study (64, cited in 37 and 61) reported changes in the normal intestinal flora of antibiotic-exposed workers. Fecal cultures from 441 workers were examined. Workers exposed to penicillins had a higher frequency of changes in bacterial flora (92%) than workers exposed to streptomycin (81%) or tetracycline (76%). There was also a clear growth of Candida and reduced amounts of vitamins C, B_1 and B_2 in their bodies.

Carcinogenicity

In 1990, the IARC classified ampicillin (trihydrate and sodium salt), with oral and parenteral administration, in Group 3: "ampicillin is not classifiable as to its carcinogenicity to humans." (28).

Dose-response / dose-effect relationships

There are no data on which to base a dose-response or dose-effect relationship for effects indicating possible Type 1 sensitization, including asthma. Shmunes *et al.* (61) reported that workers exposed to ampicillin concentrations of 0.1 to 9.9 mg/m^3 in a factory producing synthetic penicillins had a significantly higher

frequency of allergy symptoms than a comparison group exposed to less than 0.1 mg/m^3. In an unvetted report (23) a higher frequency of asthma symptoms was reported in workers exposed to 0.29 mg penicillin dust/m^3 (0.12 – 0.45 mg/m^3), measured as total dust, than in a control group. None of these studies contains clear evidence of a dose-response relationship between penicillin dust and asthma symptoms. None of them can be used to identify a level that is sensitizing.

Penicillin-induced allergic alveolitis is described in one study (13), but no exposure levels are given.

The contact-sensitizing potentials of penicillins have been tested experimentally with both humans and animals, and found to be high. Penicillins as topical medication were discontinued at an early stage because too many patients became sensitized (15).

Conclusions

Occupational exposure to penicillins via inhalation or skin contact can cause sensitization and symptoms including asthma, urticaria and anaphylaxis. There are no reliable data on exposure levels that may cause sensitization.

Occupational skin exposure to penicillins can cause allergic contact eczema. Experimental data indicate that the risk of sensitization from skin contact is high.

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Consensus Report for n-Hexanal

March 29, 2006

This document has its origin in a project described in a report from the Västernorrland County Council (45) and a subsequent publication (46). The Council report describes hexanal formation in conjunction with the production and storage of wood pellets. Other sections are based on literature published in the 2002 – 2005 period and found in searches on ToxNet (Nov. 7, 2005) and Entrez-PubMed (Jan. 20, 2006).

Chemical and physical data

CAS No:	66-25-1
Synonyms:	aldehyde C-6, caproaldehyde, capronaldehyde, 1-hexanal, hexane aldehyde, hexylaldehyde
Formula:	CH_3-$(CH_2)_4$-COH
Molecular weight:	100.18
Density:	0.814
Boiling point:	131 °C
Melting point:	-56 °C
Flash point:	32 °C
Vapor pressure:	1.5 kPa (25 °C)
Saturation concentration:	14,882 ppm (25 °C)
Solubility (water):	5.64 g/l (30 °C)
Log $P_{octanol/water}$	1.78
Conversion factors:	1 mg/m^3 = 0.245 ppm; 1 ppm = 4.1 mg/m^3

Hexanal is an aldehyde that occurs or is formed naturally in living cells of both animals and plants. Hexanal can be broken down rapidly, for example in human cells. The molecule is reactive and binds to proteins and other substances in the cells (14, 15, 33). It is attributed with irritating qualities (2). Literature that specifically describes the toxicological effects of hexanal on humans or animals is still relatively scarce, although exposure and the number of exposed people is increasing with the use of wood pellets as fuel in Sweden and elsewhere.

In an exposure chamber study, subjects noticed the smell at 2 ppm (13). For squirrel monkeys and pigtail macaques the odor threshold is below 1 ppm, and for some individuals below 1 ppb, which shows a well developed olfactory perception for hexanal and other aliphatic aldehydes (27).

Occurrence

Hexanal occurs naturally in food, and is reported to occur in at least 100 different foods (apples, strawberries, tea, tobacco, coffee etc.) (16): the article gives a maximum concentration of 300 mg/kg (3 mmol/kg). The odor of hexanal has been described as "grassy" or "leafy" (40). Hexanal has been identified in several substances, including vapors from cooking oil (50). Hexanal can be formed in foods when they spoil (35) and can occur in drinking water (0.2 – 0.8 μg/l) (26). Hexanal is used in food as a flavor additive, in organic syntheses, and in rubber, paint, insecticides etc.

A study of hexanal levels in Paris homes revealed large variations. The average value was 33.5 $\mu g/m^3$ (8.2 ppb) but the upper 95th percentile was 150 $\mu g/m^3$ (37 ppb) (7).

In a recently published study it was shown that hexanal is formed during storage of wood pellets – probably as a product of ongoing autooxidation (46). Concentrations above 24 ppm (100 mg/m^3) were measured in pellet storage areas at a factory. On a service walkway the concentration was 83 mg/m^3 and in the tractor cab at loading the measured concentration was 4.3 mg/m^3. Home heating with wood pellets is becoming increasingly popular in Sweden, and 714,000 tons of wood pellets were produced in Sweden in 2001. Levels of 0.8 mg/m^3 have been measured in basements near the furnace room in dwellings (46). Hexanal is also formed when sawdust is autoclaved for use as litter for laboratory animals (23).

Hexanal can be formed in the body tissues during lipid peroxidation (17, 33, 49). Omega-6 fatty acids give rise to hexanal. Lipid peroxidation is a pathological/toxicologic process that can be initiated in biological tissues by oxidative stress, which in turn can be initiated by several different kinds of xenobiotics. Hexanal, and other aldehydes that are formed by oxidative breakdown of polyunsaturated fatty acids, can be used as indicators that lipid peroxidation has occurred in a tissue. There are several such studies.

Hexanal has been identified in human adipose tissue, but quantitative data are not reported (36). Hexanal occurs normally in exhaled air from humans (about 18 nmol/l condensate). Smokers have higher levels, as do persons with chronic obstructive lung disease (8). Saliva also contains hexanal (1, 8, 9). Levels in blood were measured in a Chinese study: they ranged from 34 to 180 nM (3.4 – 18 μg/l) in 7 healthy subjects and from 1900 to 5500 nM in ten treated lung cancer patients (11, 28).

It may be of particular interest that high amounts of hexanal are found in atherosclerosis plaque (18), where it may play some role in the development of the disease (see below).

Exposure pathways, uptake, excretion

Occupational exposure to aldehydes can occur via inhalation, skin uptake and oral uptake (38). The primary exposure pathways for most of the population are probably inhalation and intake in food and water. The average intake of hexanal

for a Dutch population was estimated to be 16.5 μg/day (6.0 mg/year) (21). No measurements of uptake were found.

When mice were given intravenous injections of hexanal (68 mg/kg) plasma levels dropped within a few minutes but then remained fairly stable (at about 170 nmol/ml) for two hours. In lungs the concentration peaked within 30 minutes and then began to drop (49).

Hexanal levels in 24-hour urine samples from healthy controls were around 2 μmol (200 μg) (37), and did not increase with an hour of physical exercise.

Metabolism

No *in vivo* studies of metabolism were found. In general, aldehydes can be broken down to the corresponding acids via metabolic oxidation. The process can be catalyzed by aldehyde dehydrogenase (ALD). It was shown in an *in vitro* model that ALD protects the cells against hexanal-induced inhibition of cell growth (47), and a form found in humans has been found to be highly active. In an earlier study it was shown that hexanal was oxidized by another isoform of ALD, which is induced by TCDD (2,3,7,8-tetrachloro-dibenzo-p-dioxin) and expressed in rat liver tumors (31).

No effects on glutathione levels in mouse lungs were seen after injection of 68 mg hexanal/kg b.w. (49).

Reduction of hexanal to hexanol has been observed in rat brain, and it has also been shown that hexanal can be condensed with pyruvate to acyloins and 3-hydroxyoctane-2-one by the activity of pyruvate dehydrogenase (24).

Toxicity

There are no long-term toxicity studies of hexanal.

Human data

Low-molecular aldehydes in general are reported to be strongly irritating to mucous membranes in nose, mouth and upper respiratory passages. No specific information on the effects of the longer-chain hexanal on humans was found in *Patty's Industrial Hygiene and Toxicology* (2).

In one study, volunteers (21 – 28 years old) were exposed to 0, 2, or 10 ppm hexanal for two hours on three occasions at least 2 weeks apart (13). They were not told the order of the exposures. They graded their symptoms before, during and after the exposures by markings on ten 10-cm visual analog scales (VAS), one for each symptom group. The scales were pre-printed with descriptions of increasing severity. The ten symptom groups were: "eye discomfort: burning, irritated or running eyes; nose discomfort: burning, irritated, or runny nose; throat or airway discomfort; breathing difficulty; solvent smell; headache; fatigue; nausea; dizziness; feeling of intoxication".

Lung function, nasal swelling, blinking frequency and inflammation markers (CRP and IL-6) in blood samples (taken 3 hours after the end of exposures) were also measured. At 10 ppm the subjects reported (in addition to solvent smell) eye discomfort (16.5 mm/100 mm) and other symptoms (16.5 mm on the VAS did not exceed the verbal rating "somewhat"). Blinking frequency was also significantly higher at 10 ppm. The authors concluded that 10 ppm hexanal has a weak irritating effect, and in this study it was the LOAEL (lowest observed adverse effect level) and 2 ppm was the NOAEL (no observed adverse effect level).

Animal data

The LD_{50} for oral administration of hexanal to rats is reported to be 4890 mg/kg (41).

In an inhalation study from the 1950s (41), rats were exposed to 2000 ppm hexanal for 4 hours. One of 6 died. In the same study rats were exposed to "concentrated vapor" for 1 hour and mortality was then 0/6. Hexanal is reported to be slightly irritating to the eyes and skin of rabbits.

In Grant's *Toxicology of the Eye* (20), hexanal is ranked 5 on a 10-point scale for eye irritation in rabbits.

The RD_{50} (the concentration that reduces respiratory rate by 50%) for hexanal inhalation was 1116 ppm for $B6C3F_1$ mice and 1029 ppm for Swiss-Webster mice (42).

In a large study, male and female rats were given hexanal in drinking water (calculated intake 0.1, 1.2, 12.6 or 124.7 mg/kg/day) for 4 weeks. There were 10 males and 10 females in each dose group. There were also 2 control groups in the study, as well as groups of rats exposed to other substances. Organ weights, 18 hematalogical parameters and liver microsome activities were measured. Histological examinations were made of 26 organs/tissues, including pancreas. All the rats survived and their growth was not affected at any dose level. The authors report that treatment-related morphological changes (slight effects on thyroid, liver and kidneys) occurred only in the high-dose group. There was also a slight reduction of lactate dehydrogenase activity in serum, but it was not dose-dependent (26).

Hexanal emitted from autoclaved sawdust in cages for laboratory animals has been shown to contribute to deaths in pups of genetically modified mice. One explanation may be that hexanal (4 μg/35 g sawdust) induces Fos expression (increases the activity of protein that increases cell growth) in certain brain areas in the dams. This might affect maternal behavior and have an adverse effect on nursing (22). The exposure level is unknown, as is the relevance to humans.

In vitro data

There are quite a few published studies in which hexanal was tested in various cell models. A representative selection is reviewed below.

In a study with cultured human endothelial cells from umbilical veins, hexanal was not toxic (concentrations up to 0.1 mM) in relation to unsaturated aldehydes

and especially in relation to linoleic acid hydroperoxide (25). At concentrations between 10 and 30 mM hexanal a large portion of primary hepatocytes, from both rats and humans, died (trypan blue exclusion test). All the rat hepatocytes died at a concentration of 100 mM (32). Growth of fibroblasts from Chinese hamsters (V79) was inhibited by 0.1 mM or more (47). Formation of the inflammatory signal substance TNF-α was inhibited in human macrophages by lipid peroxidation products. Hexanal was one of the less potent inhibitors, but concentrations of 0.2 to 1.0 mM had some effect (19). Hexanal (50 μM) increased expression of the CD36 gene in macrophages (48). In comparisons with other lipid peroxidation products, hexanal has a milder but still toxic effect, for example by reducing ATP levels (4, 12).

In a study in which β-cell islands isolated from rat pancreas were exposed to various combinations of inflammatory cytokines, hexanal was found to be one of the substances formed in these cells in response to oxidative stress. It was also shown that low concentrations of hexanal (0.001 – 0.2 mM) reduced insulin production and damaged the cells (44). In a later study (34) on a similar model it was shown that hexanal (0.1 mM) yielded a fairly weak effect on insulation production (in comparison to other lipid peroxidation products). The effect reported at the lowest concentration used by Suarez-Pinzon *et al*. (44) was not confirmed. A search (November 5, 2004) in the cited articles gave no indication that hexanal's toxicity to β-cell islands was studied further.

Smooth muscle cells from human umbilical cord were used in a model focused on cell communication (gap-junction intercellular communication, GJIC) and atherosclerosis. A dose-dependent inhibition was induced by a potent lipid peroxidation product, and hexanal also had some effect. The effect was not dose-dependent, however, and GJIC was only slightly reduced in the dose interval 0.005 – 0.05 mM (10).

Hexanal has been identified in plaque from sclerotic blood vessels (18). It has also been shown that the cholesterol-reducing medicine simvastatin reduces the amount of hexanal in the plaque, raising the question of whether hexanal and other lipid peroxidation products may play some role in atherosclerosis. In further studies, the group examined the induction of the glycoprotein TF (tissue factor), via Fos activation, in smooth muscles from blood vessel walls (5). It was found that 5 μM hexanal was a powerful inducer of TF. Earlier studies reported that TF can stimulate blood clotting after e.g. plaque rupture (43), and the authors (5) conclude that endogenously formed hexanal may function as a signal substance that can contribute to the occurrence of heart infarct, stroke etc.

Genotoxicity, carcinogenicity

The effects of various aldehydes on DNA cross-linking and single-strand breaks (SSB) were studied using V79 cells (29). Hexanal (0.5 – 4.5 mM) was one of the substances inducing single-strand breaks. Two of the same authors are in a group that later published further genotoxicity studies (3), this time on resistance

development in V79 cells to 6-thioguanine or ouabain (as measures of genotoxicity). They concluded that hexanal is mutagenic in concentrations around 3 – 30 mM. In subsequent studies with hepatocytes from rats and humans (32) it was shown that 30 mM has some effect on DNA (unscheduled DNA synthesis, UDS) in rat hepatocytes, but at this concentration half of the cells died. Fewer of the human cells died, and elevated UDS was not seen. Other weaknesses of the studies are that only two cell batches were used and that the limit for a clear positive response was not reached. The group has now concluded that the probability of genotoxic effects on humans is negligible: that hexanal is at most weakly genotoxic and that damaging concentrations are highly unlikely to occur in human tissues. They were probably referring primarily to endogenous hexanal, but inhaled hexanal probably can not generate millimolar concentrations in cells (e.g. nasal mucosa) either.

A Salmonella strain (TA104) that is particularly sensitive to lipid peroxidation products was used in a genotoxicity study: hexanal was not mutagenic (30). A risk assessment survey on the carcinogenic potential of aldehydes (16) makes no reference to the above publications on genotoxicity, and treats hexanal as a substance for which there is no relevant data.

No carcinogenicity studies were found.

Effects on reproduction

In an *in vitro* study with human spermatozoa, hexanal was shown to inhibit fructose metabolism. The inhibiting concentration was about 0.3 mM (39). In a later study of spermicides (6) it was shown that the mobility of the sperm was inhibited at a hexanal concentration of 0.1% (= 1 g/liter; 10 mM).

Dose-effect / dose-response relationships

Earlier, non-quantitative data indicate that hexanal can be irritating to mucous membranes and skin. A recently published exposure chamber study with healthy subjects reports uncertain effects at 2 ppm and irritation, including increased blinking frequency, at 10 ppm (13).

The RD_{50} (the concentration that reduces respiratory rate by 50%) for hexanal has been reported to be about 1000 ppm for mice (42). RD_{50} is used as a measure of sensory irritation in airways. The ACGIH threshold limit values based on irritation are generally about 3% of the RD_{50} value. For hexanal this would be about 30 ppm.

There are no long-term animal studies of inhalation exposure, but a study in which animals (4 dose groups) were given hexanal in drinking water reports a LOAEL (slight morphological changes in thyroid, liver and kidneys) of 125 mg hexanal/kg b.w./day (26).

In vitro studies indicate that hexanal is relatively non-toxic to many types of cells. In most of the studies the lowest concentration yielding cell death is a bit below 1 mM (about 100 mg/l), a concentration that may have been reached with

the highest dose (1000 mg/l) in the drinking water study (26). One cell study, however, found that hexanal can be toxic to the insulin-producing cells in the pancreas at concentrations as low as 0.001 mM (44). It is possible that these cells are particularly sensitive to hexanal, but in the drinking water study there were no reported effects on parameters such as blood glucose after the 4 weeks of exposure. It can also be noted that no confirmation or commentary on this finding was found in a search (November 4, 2005) of subsequently published literature.

A recently described effect of hexanal pertains to its possible role in atherosclerosis and its complications (5). In a cell model, it was shown that 5 μM functions as a signal mediator and may contribute to blood clot formation. The relevance *in vivo* is unknown, as is the air concentration that can yield sufficiently high blood content.

Early *in vitro* studies reported that hexanal could be genotoxic. It should be pointed out that the concentrations were high and the effects small, and that the results could not be confirmed (according to the literature reviewed for this Report) by other laboratories (30). In the latest work on hexanal published by this research group, the authors themselves conclude that hexanal's possible genotoxicity is negligible for humans (32), (see Table 1).

Conclusions

There are no long-term studies, and the available information is not sufficient to establish a critical effect of occupational exposure to hexanal. Exposure chamber studies have shown that hexanal is irritating at an air concentration of 10 ppm.

Table 1. Dose/concentration – effect relationships for exposure to hexanal.

Type of study	Exposure	Effects	Ref.
Human study			
Exposure chamber study	2 ppm (8 mg/m^3) 2 hours	No clear effects	13
	10 ppm (40 mg/m^3) 2 hours	Irritation with increased blinking frequency	
Rat study			
Hexanal in drinking water	12,6 mg/kg/day 4 weeks	NOAEL for most endpoints	26
	125 mg/kg/day 4 weeks	Slight effects on thyroid, liver, kidneys	
Mouse study			
Inhalation	1116 ppm (4.6 g/m^3) (Swiss-Webster) 1029 ppm (4.2 g/m^3) (B6C3F$_1$) 10 minutes	RD$_{50}$ (the concentration that reduces respiratory rate by 50%)	42
In vitro studies			
β-cells (rats)	0,001 mM	LOAEL for reduced insulin production (results not confirmed, and contradicted by other data)	44
Human vascular smooth muscle cells	0,005 mM	Induction of TF	5
Hamster V79 fibroblasts	0,1 mM	Inhibited growth	47
Human spermatozoa	0,3 mM	Inhibited fructose metabolism	39
Mutagenicity studies	3-30 mM	Slight mutagenicity with high cytotoxicity; dubious value	32

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Consensus Report for Nitrous Oxide (laughing gas)

June 7, 2006

This report is based primarily on a criteria document (28) compiled at the request of the Swedish Criteria Group for Occupational Standards, supplemented by a review of recent literature. It updates the consensus report published in 1981 (39).

Chemical and physical data. Uses

CAS No.:	10024-97-2
EG No.:	233-032-0
Synonyms:	nitrous(I)oxide, dinitrogen oxide, laughing gas
Earlier name:	nitrogen oxidul
Formula:	N_2O
Molecular weight:	44
Density (kg/m^3):	1.98 (0 °C, 1 bar)
Relative density:	1.5 (air = 1.0)
Boiling point:	- 88.5 °C
Conversion factors:	1 ppm = 1.83 mg/m^3 (20 °C, 101.3 kPa) 1 mg/m^3 = 0.55 ppm (20 °C, 101.3 kPa) 1% = 10,000 ppm
Other data:	1 liter of liquid yields 662 liters of gas at atmospheric pressure and a temperature of 15 °C. The gas is heavier than air; there is a risk that it will accumulate in enclosed spaces. Oxidating. The gas does not burn but supports combustion.

Nitrous oxide (N_2O) is a colorless gas with a faint, sweetish odor. It is used as an anesthetic. It is popularly called laughing gas because of its ability to produce euphoria.

Occurrence in the workplace

Nitrous oxide was first used as an anesthetic in 1844 (48). It was introduced in Sweden in the 1860s, and is still a basic component in most combination anesthesia procedures.

Occupational groups exposed to nitrous oxide are operating room staff, midwives, dental surgeons and their assistants. With modern methods of anesthesia administration, levels in ambient air are generally well below 400 ppm (732 mg/m^3) (35, 37). With older administration methods and in poorly ventilated operating rooms, levels of 400 – 3000 ppm (732 – 5490 mg/m^3) have been registered, with a few peaks as high as 6000 ppm (10,980 mg/m^3). For setting exposure limits, however, the studies of greatest relevance are those of lower exposures.

Nitrous oxide is also used as motor fuel, but exposure from this use is not relevant in the present context.

Uptake, biotransformation, excretion

Nitrous oxide has long had widespread use as an anesthetic, and the pharmacokinetics of inhalation have therefore been thoroughly studied: they are well described by physiologically based pharmacokinetic (PBPK) models (see for example Reference 44). Nitrous oxide is a small molecule that diffuses rapidly through cell membranes, and it is therefore rapidly taken up in the lungs and other body tissues. Since solubility in blood and fat is low, with coefficients of 0.42 for blood/air (47) and 1.4 for fat/air (57), accumulation in the body, including fatty tissue, is insignificant, and — in contrast to many solvent vapors — increased workload increases body burden only slightly. After exposure is stopped the concentration of nitrous oxide in blood drops rapidly, with a half time of about a minute. The remaining concentration then drops more slowly, with a half time of about 20 minutes (calculated from information in Reference 44).

Nitrous oxide is excreted in urine, and there is a good correlation between concentrations measured in air and excreted N_2O (35, 38, 56). Biological monitoring of nitrous oxide has therefore been proposed as an alternative to air monitoring for the purpose of risk assessment. Urine samples for this purpose should be taken both before and after exposure.

No studies on the metabolism of nitrous oxide were found.

Toxic effects

Effects on blood-forming organs

Animal experiments

In experiments in which rats were exposed to high concentrations of nitrous oxide, 20% (200,000 ppm; 366,000 mg/m^3) or more, blood profiles were affected. Rats exposed to 1% nitrous oxide (10,000 ppm; 18,300 mg/m^3) 6 hours/day, 5 days/week for up to 6 months (to resemble the occupational exposure of a dental surgeon) had no observable changes in bone marrow, but they had significantly lower hemoglobin levels (21).

Human data

Patients treated by breathing a mixture of 50% oxygen and 50% nitrous oxide constantly for 14 to 17 days developed several morbid changes in their blood profiles (42). The authors point out that continuous N_2O treatment can result in acute bone-marrow aplasia (loss of blood-forming function). The changes are reversible, however, with return to normal blood profile when the treatment is stopped.

Similar results were obtained in another study of patients who were treated with 50% oxygen and 50% N_2O for either 24 hours or 5 to 12 hours (2). Nitrous oxide interacts with vitamin B_{12}-dependent enzymes, which may result in megaloblastic anemia (characterized by abnormally large erythroblasts – immature red blood cells) (2). This interaction may also lie behind the occurrence of myeloneuropathy (neural pain, deterioration of reflexes in arms and legs), see below .

In one study (60), 21 dentists (volunteers) were followed for 3 to 11 weeks. They wore personal monitors while they were doing work involving exposure. Bone-marrow samples were taken and other studies were also made during this period. The time-weighted average exposures to N_2O ranged from 159 to 4600 ppm (291 – 8418 mg/m^3) and reported exposure times ranged from 0.5 to 27 hours per week. Blood tests, including B_{12} and folate, were normal, as were the neurological tests. However, results of a deoxyuridine inhibition test (a sensitive test for inhibition of DNA synthesis) were pathological in two and borderline in one of the 20 subjects given this test. In the two subjects with pathological results, profiles of peripheral blood showed hypersegmented neutrophils with more than five nuclei, and in bone marrow there were giant metamyelocytes and slight melanoblastic changes. There was no clear correlation between exposure (ppm x hours) and effects. In the three with abnormal test results, the lowest exposure level at which effects were noted was 1800 ppm (3294 mg/m^3) and this dentist had been exposed for 27 hours per week. The other two had exposures of 1900 and 2500 ppm (3477 and 4574 mg/m^3) for 6 and 10 hours per week respectively. The three dentists with abnormal inhibition tests were among the six subjects who had exposures above 10,000 ppm x hours per week. The authors concluded that their study strengthens suspicions that N_2O interferes with the metabolism of vitamin B_{12}.

Effects on the peripheral nervous system

Persons occupationally exposed to nitrous oxide in poorly ventilated dental offices, or those who abuse the substance for 3 months or longer, have developed tingling and numbness in extremities, reduced ability to register sensory impressions (especially touch), balance problems etc. Measures of neural conductivity yielded lower values, possibly due to nitrous oxide's interference with vitamin B_{12}-dependent metabolism (43). Since there are no quantitative exposure data, no dose-response relationships can be given.

There are still people who abuse nitrous oxide, and a few case reports have been published. Pema *et al*. (50) describe a case of myelopathy compatible with

vitamin B_{12} deficiency in a 31-year-old man who had abused nitrous oxide for several years.

Effects on the central nervous system

Several laboratory studies have confirmed that nitrous oxide in high doses has CNS and psychological effects.

Thirty volunteers (male undergraduates) were exposed to either 500 ppm (915 mg/m^3) nitrous oxide or air for four hours, and then given function tests (14). Each person was exposed on one occasion to nitrous oxide and on the other occasion to air alone. The subjects were exposed in an exposure tent, and the tests were given immediately afterward. On the group level the only difference between the nitrous oxide and the air exposure was a lower score on a short-term memory (digit-span) test.

The same researchers (15) also studied the effect of four hours of exposure to 50 ppm (91.5 mg/m^3) nitrous oxide. Twenty volunteers (male undergraduates) were exposed by breathing through a mask. Ten were exposed first to nitrous oxide and a week later to air, and the other ten were exposed in reverse order. The psychometric tests were begun after two hours of exposure and the last one was given immediately after exposure ended. At the group level, test results after the nitrous oxide exposure were significantly worse on the 3-minute and 7-minute audiovisual tests (tests of perception and reaction time), given respectively about 2.75 and 4 hours after the start of the exposure. Another group of 20 persons, exposed additionally to 1 ppm halothane, had significantly worse results in two other tests and were better on one test than the group exposed to nitrous oxide alone.

These results could not be confirmed in other studies (24, 32, 55); however, these later studies are not directly comparable because the exposures were shorter.

In a similarly designed study (63), 24 volunteers were exposed to either air or 50 ppm (91.5 mg/m^3) nitrous oxide in an exposure chamber for 4 hours. Each person was tested twice, once with and once without nitrous oxide exposure. The psychometric tests were given during the last 40 minutes in the exposure chamber, and included an audiovisual task test like the one that showed effects in the study by Bruce and Bach (15). No differences between the nitrous oxide and air exposures were observed, either in that test or in tests of simple reaction time, four-choice reaction time, or stressalyzer (63).

Bruce has since published an article (17) in which he states that he does not consider his results to be valid. “There is no longer any need to refer to our conclusions as controversial. They were wrong, derived from data subject to inadvertent sampling bias and not applicable to the general population. The NIOSH standard should be revised.” In addressing the issue of comparability, internal and external validity, Bruce (16) suggests that the subjects of his studies, male Mormons, are unusually sensitive to such substances as anesthetic gases because of their lifestyle. The external validity of the results can therefore legitimately be questioned. Neither he nor anyone else has criticized or questioned the

methodology of his studies, and the "sensitive subjects" argument may explain why other researchers could not confirm the results reported by Bruce and Bach.

Ayer *et al*. (6) studied 10 dental assistants at work. They were tested before they began work, after 20 minutes of anesthetic administration, and immediately after they had finished administering the anesthetic. The assistants were exposed to an average N_2O level of 210 ppm (383 mg/m^3) during the 35-minute sessions. The tests used were the color-naming test, pegboard test and fusion-frequency of flicker test. None of the tests showed any significant differences between results obtained before work, after 20 minutes of anesthetic administration or after the end of the exposure. Twenty dentists were also studied. Ten of them used nitrous oxide at work: their average exposure was 536 ppm (981 mg/m^3), range 150 – 1500 ppm (274.5 – 2745 mg/m^3). They were compared with ten unexposed dentists (5). All of them were tested before beginning to treat a patient, 20 minutes after the treatment had begun, and immediately after the treatment was completed. The length of the treatment periods is not reported. The exposed dentists and the unexposed dentists had equivalent results on the tests they were given: the Lafayette pegboard test and the Ruesh color naming test. The authors concluded that their results were in agreement with earlier reports on effects of low concentrations of nitrous oxide on psychomotor activity, i.e. that no effect can be demonstrated, but they mention the possibility that several years of exposure may have negative consequences.

Lucchini *et al*. (46) published a study suggesting that exposure to low concentrations of nitrous oxide, geometric means for a workweek 50.9 ppm (93.1 mg/m^3) to 54.2 ppm (99.2 mg/m^3), yields measurable, reversible effects on reaction time. The studied group comprised 30 doctors and nurses who worked in operating rooms for heart surgery. They were followed for a week with exposure to nitrous oxide and for a week without nitrous oxide exposure. Two exposure-free weeks passed between the two studied weeks. A group of 20 randomly chosen nurses and doctors who worked in other parts of the same hospital were used as controls. The exposed group and the control group were comparable with regard to sex ratio, age, alcohol consumption and smoking habits. Exposure to nitrous oxide was measured in the breathing zone with passive dosimeters during the three-hour exposures. The measured effects were simple reaction time and serum prolactin and cortisol. When the exposed group was compared with controls, there were significant differences in simple reaction time and serum prolactin measured after work on the last day of the workweek. For the exposed group there was also a significant difference between results after working for a week with exposure and results after the unexposed week. The study reports only exposure to nitrous oxide, but the exposures probably included halogenated anesthetic gases. The authors themselves do not attribute the observed effects to nitrous oxide exposure, but to "exposure to anesthetic gases."

There are a few other studies using psychometric tests to quantify effects of exposure. However, they report such high exposure levels – 30,000 ppm (54,900 mg/m^3) or above – that they provide no useful information (3, 20, 26, 30, 62).

There is a Swedish study reporting an elevated risk of multiple sclerosis in nurse anesthetists (31). The study covers 90 nurses with MS, identified via ads in professional journals (*Vårdfacket* and *Reflex*). Exposures were determined by questionnaire and are described only briefly in the article ("...they were exposed to a wide spectrum of anaesthetics..."). No conclusions on possible effects of nitrous oxide exposure can be drawn from this study.

Other effects

In a few epidemiological studies (48), elevated incidences of liver and kidney diseases have been observed among anesthesia personnel (more than one year of occupational exposure). These studies provide no information on exposure levels, and it is probable that the exposures also involved anesthesia gases other than nitrous oxide.

In animal experiments, exposure to nitrous oxide is at such high levels – above 70,000 ppm (128,100 mg/m^3) – that the results are irrelevant in a discussion of occupational exposure limits.

Mutagenicity, genotoxicity, carcinogenicity

Animal experiments

Nitrous oxide was not mutagenic in Ames' tests, either with or without metabolic activation and at up to 5 atm overpressure (5 x atmospheric pressure), in various strains of *Salmonella typhimurium* (7, 9, 67). Nitrous oxide, either alone or in combination with halothane, was not mutagenic in tests with Chinese hamster lung fibroblasts (V79 cells) (59). However, nitrous oxide did increase the number of nuclei with halothane-induced abnormalities (tripolar and tetrapolar spindles, double or triple nuclei, micronuclei) in this type of cell (58). Nitrous oxide, either with or without addition of metabolizing systems, induced no sister chromatid exchanges in Chinese hamster ovary (CHO) cells (68). A study from 1974 reports a weak positive result in a sex-linked recessive lethal test on fruit flies (*Drosophila melanogaster*) (33). Later studies with the same test system have been negative (40), even when the nitrous oxide was tested in combination with halothane, enflurane or isoflurane (11). Nitrous oxide is weakly mutagenic to Tradescantia, and it has been shown to cause aneuploidy and polyploidy (deviant and multiple chromosome counts) in other plants when they are exposed with overpressure. It also potentiates the mutagenic effects of ionizing radiation in cell cultures (8).

There are a few carcinogenicity studies of nitrous oxide (10, 22, 29). Although all of them were negative, they are too few to allow any definite conclusions on carcinogenicity.

Epidemiological studies

Several epidemiological studies have reported elevated frequencies of cancer, especially in lymphatic tissue, among anesthesia personnel (27). None of these

studies contains information on exposure times or exposure levels. Nor can it be determined whether these persons were exposed to nitrous oxide alone or in combination with other anesthetic gases. There are also studies that do not indicate elevated cancer frequencies in association with exposure to anesthetic gases. In the study described below (23), no significant increase of total cancer frequency could be shown for dentists and their assistants. There was a significantly higher frequency of cervical cancer among the female assistants with high exposure, but exposures were not reported.

A good many of the articles on anesthetic gases published in the past few years have been genotoxicity studies. There are several studies reporting sister chromatid exchanges (SCE), micronuclei and chromosome aberrations in exposed personnel (19, 36, 41, 45, 49, 53, 54). The difficulties with these studies are that the exposure is rarely to nitrous oxide alone and that none of them gives exposure levels. They thus provide no information that can be used in establishing either a critical effect or a risk level for occupational exposure to nitrous oxide. In the study by Hoerauf *et al*. (36) there was simultaneous exposure to nitrous oxide and isoflurane. The reported 8-hour time-weighted averages were 11.8 ppm (21.6 mg/m^3) and 0.5 ppm (0.9 mg/m^3). The frequencies of SCE in 27 exposed anesthesiologists and 27 unexposed doctors were compared. All were non-smokers. There was a statistically significant difference between the two groups. The difference was still significant ($p = 0.03$) when exposed men ($n = 13$) were compared to unexposed men ($n = 18$), but there was no such difference for the women.

Lewinska *et al*. (45) studied 46 female scrub nurses exposed to nitrous oxide as well as sevoflurane and isoflurane. The two latter gases were not monitored but it was reported that they did "..not exceed the adopted TLV of 18 mg/m^3". Nitrous oxide in the breathing zone was in the range 36 – 2308 mg/m^3 (19.7 - 1261 ppm). The controls were 28 female nurses working in the same hospital but not exposed to anesthetic gases or other suspected genotoxic substances. The exposed subjects had significantly higher numbers of micronuclei, and linear regression analysis showed correlations between the occurrence of micronuclei and both exposure to nitrous oxide ($r = 0.6$; $p = 0.00007$) and number of years in exposed work ($r = 0.6$; $p = 0.0001$). The correlations were based on exposure levels above 500 mg/m^3 and more than 20 years of exposure, respectively. Regression analysis also showed a correlation between exposure level and frequency of micronuclei. Fluorescence *in situ* hybridization (FISH) analysis of chromosome aberrations indicated a pro-aneugenic effect (aneuploidy).

Effects on reproduction

Animal experiments

Pregnant rats were exposed to 100, 1000 or 15,000 ppm (183, 1830 or 27,450 mg/m^3) nitrous oxide, either 8 or 24 hours/day for 4 to 10 days during the second and/or third week of gestation, and then compared with control groups (25). In the

two higher dose groups fetal death rates and pregnancies/rat rates where significantly different from the controls. In one of three groups exposed to 100 ppm (183 mg/m^3) there was a suggestion of elevated fetal mortality, but there was no adequate control group.

Rats were continuously exposed to 0.5% (5000 ppm; 9150 mg/m^3) nitrous oxide on days 1 to 9 of gestation and then killed: compared to the controls there were significantly smaller litter sizes and higher proportions of resorptions. Fetuses in the exposed group were significantly smaller and had more skeletal anomalies compared to the unexposed group (64).

Pregnant rats were exposed to 0.1%, 0.05% or 0.025% (1000, 500 or 250 ppm; 1830, 915 or 457.5 mg/m^3) nitrous oxide until day 19 of gestation, when they were killed. The exposures had the following results (65): In the group with the 0.1% exposure there was a statistically significant reduction in the number of living fetuses, an increase in the proportion of resorptions and in fetuses a higher proportion of skeletal anomalies, compared with the other groups. No difference in fetal body weight or sex ratio was observed. The authors concluded that with constant exposure the critical concentration of nitrous oxide that can cause fetal death in rats is between 500 and 1000 ppm (915 – 1830 mg/m^3).

Vieira *et al*. (66) performed another experiment similar to the one described above (65). Here, however, exposures were intermittent – 6 hours/day for 19 days – and exposure levels were 250, 500, 1000 or 5000 ppm (457.5, 915, 1830 or 9150 mg/m^3). The rats were then killed. There were 12 pregnant rats in each exposure group. No unexposed group is mentioned. In the group exposed to 5000 ppm (9150 mg/m^3) there was a statistically significant reduction in the number of living fetuses, but there were no other differences between the groups. The authors interpret this observation with reference to their earlier study, and draw the conclusion that with intermittent exposure the risk of fetal effects is lower than with constant exposure at the same exposure levels.

Data from epidemiological studies

Many studies in various countries have reported elevated risk of miscarriage in personnel occupationally exposed to anesthesia gases. These studies, however, contain too little information on type or level of exposure to be useful for assessing the health effects of occupational exposure to nitrous oxide.

A meta-analysis of studies reporting spontaneous abortions in women exposed to anesthetic gases concludes that epidemiological studies indicate an elevated risk (13). The analysis was based on a review of 19 published studies, 2 of which were case-control studies. There was no information on either the type(s) of anesthetic gas or the exposure levels.

Two other survey articles (18, 61) on risks of occupational exposure to anesthetic gases present the conclusion that the literature does not provide adequate support for asserting that exposures cause miscarriages or birth defects. One article (61) reviews 14 studies published in the 1971 – 1982 period, and the

other (18) reviews 28 articles published in 1973 – 1996. Neither of them contains information on either type(s) of gas or levels of exposure.

In a Canadian study (34), data from 8,052 persons exposed to anesthesia gases and 2,525 unexposed persons were collected by questionnaire. There was an elevated incidence of spontaneous abortion among the exposed women. No information on types of gas or exposure levels is given.

From the results of a large epidemiological study of dentists and dental assistants in the United States (in which attrition was about 20%), the authors conclude (23) that long-term exposure of male dentists to anesthetic gases leads to an elevated frequency of miscarriage in their wives, and that exposed female assistants have elevated frequencies of miscarriages and children with congenital defects. The dental assistants who were exposed to nitrous oxide alone had twice as many miscarriages and half again as many children with congenital defects as the unexposed mothers.

More recent studies have focused on the question of reduced fertility in female dental assistants exposed to nitrous oxide or to anesthetic gases in general. In an American study (51) covering 7,000 female dental assistants exposed to nitrous oxide or to combined anesthesia gases, telephone interviews were used to collect information on exposure and time to pregnancy. An effort was made to differentiate high from low exposure by asking whether there was exhaust ventilation. No exposure levels are given. There was longer time to pregnancy in a group of 19 women described as highly exposed (no exhaust and more than 5 hours of exposure per week). Similar data were presented by Ahlborg *et al*. (1) in a study of midwives, in which 84% of 3,985 midwives responded to a questionnaire on working conditions, exposures and pregnancy. There is no information on exposure levels. The authors concluded that shift work has a negative effect on fertility. Regarding effects of nitrous oxide, there were no noteworthy observations except for reduced fertility in a small group of midwives who assisted at more than 30 births per month. None of these studies gives exposures other than those reported by the respondents in the form of time.

A large study of female dental assistants in the U.S., the same study material and design as Rowland *et al*. 1992 (51), reports a more than doubled risk of spontaneous abortion (RR = 2.6; 95% CI 1.3 – 5.0) for women occupationally exposed to nitrous oxide for 3 or more hours per week. No data on exposure levels are given: exposure is described only as 3 or more hours of work per week in rooms without exhaust ventilation (52).

In a study by Axelsson *et al*. (4), based on the same material used by Ahlborg *et al*. (1), it was found that the risk of spontaneous abortion was higher for shift workers and night workers than for those working days. No correlation was found between spontaneous abortions and exposure to nitrous oxide. A third publication based on this material reports inhibition of prenatal growth and lower birth weights (average 77 grams) in children whose mothers were exposed to nitrous oxide during pregnancy (12). None of these studies gives exposure levels; the only exposure information is that provided by the subjects in the form of estimates of exposure time.

In summary, there is a picture of elevated risk of spontaneous abortion with occupational exposure to nitrous oxide, but it is difficult to assess. Early studies show an elevated risk and more recent studies report little or no risk. One explanation – aside from purely methodological sources of error – can be that the studies reflect different exposure conditions: changes in work methods introduced in the 1980s and 1990s have reduced exposure levels and thus also reduced risks.

Dose-effect/dose-response relationships

There are few studies of occupationally exposed persons in which exposure was restricted to nitrous oxide alone, and even fewer containing quantitative data on exposure. Epidemiological studies have suggested that exposure to nitrous oxide may be associated with toxic effects on reproduction (reduced fertility) (1, 51), but dose-response relationships are poorly known.

Effects on reproduction of laboratory animals are summarized in Table 1. The lowest exposure level at which statistically significant embryotoxic effects have been observed in rats is 1000 ppm. One study reports elevated fetal mortality with exposure to 100 ppm, but there are no adequate control group.

Acute effects on mental function with exposure to low concentrations of nitrous oxide are summarized in Table 2. Disturbances of mental function were reported in an experimental study with exposure to 50 and 500 ppm (91.5 and 915 mg/m^3) for four hours. The observations were not confirmed in other studies with exposures up to 4000 ppm for 30 minutes, and the author himself later retracted his conclusions.

Conclusions

There are no data from which to determine a critical effect of occupational exposure to nitrous oxide.

Judging from animal experiments, the critical effect of exposure to nitrous oxide is its toxic effect on reproduction. The lowest exposure level at which statistically significant embryotoxic effects are seen in rats is 1000 ppm (1830 mg/m^3). Occupational exposure has been shown to affect fertility, but in these studies little is known about dose-response relationships.

When discussing an occupational exposure limit for nitrous oxide it has to be taken into consideration that co-exposure to other anesthetic gases is common and that joint effects may occur.

Table 1. Embryotoxic/fetotoxic effects observed in rats experimentally exposed to low concentrations of nitrous oxide (≤ 5000 ppm; 9150 mg/m^3).

Exposure (ppm)	Time	Effects	Ref.
5000	24 hours/day, days 1–19 of gestation	Statistically significant increase in resorptions, fewer pregnancies, higher incidences of skeletal anomalies, inhibited fetal growth	64
5000	6 hours/day, 5 days/week, days 1–19 of gestation	Significant reduction in numbers of living fetuses	66
1000	24 hours/day, days 12–19 of gestation	Statistically significant reduction in pregnancies and higher fetal mortality	25
1000	8 hours/day, days 10–13 of gestation	Statistically significant increase in fetal mortality	25
1000	24 hours/day, days 1–19 of gestation	Statistically significant reduction in number of living fetuses, increased incidence of resorptions and skeletal anomalies	65
500	24 hours/day, days 1–19 of gestation	No fetotoxic effect	65
250	24 hours/day, days 1-19 of gestation	No fetotoxic effect	65
100	8 hours/day, days 10–13 of gestation	Elevated fetal mortality (adequate controls lacking)	25

Table 2. Acute effects on mental function observed with exposure to low concentrations of nitrous oxide.

Exposure (ppm)	Time (minutes)	Dose (ppm x min.)	Number of subjects	Effects	Ref.
4000	30	120 000	10	No effect on mental function*	24
2000	30	60 000	10	No effect on mental function*	24
1000	30	30 000	10	No effect on mental function*	24
536 (average)	– **	– **	10 10 controls	No effect on mental function of dentists during work*	5
500	240	120 000	30	Statistically significant changes in mental function	14
210 (average)	35	7350	10	No effect on mental function of dental assistants during work*	6
50	240	12 000	20	Statistically significant deterioration of mental function	15
50	240	12 000	24	No effect on mental function*	63

* Applies only to the test systems used.
** No information given.

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Summary

Montelius J (ed). *Scientific Basis for Swedish Occupational Standards*. XXVII. Arbete och Hälsa 2006:11:1-64. National Institute for Working Life, Stockholm.

Critical review and evaluation of those scientific data which are relevant as a background for discussion of Swedish occupational exposure limits. This volume consists of the consensus reports given by the Criteria Group at the Swedish National Institute for Working Life from October, 2005 through June, 2006.

Key Words: Ammonia, n-Hexanal, Laughing gas, Nitrous oxide, Occupational exposure limit (OEL), Penicillins, Risk assessment, Scientific basis, Toxicology.

Sammanfattning

Montelius J (ed). *Vetenskapligt underlag för hygieniska gränsvärden*. XXVII. Arbete och Hälsa 2006:11:1-64. Arbetslivsinstitutet, Stockholm.

Sammanställningar baserade på kritisk genomgång och värdering av de vetenskapliga fakta, vilka är relevanta som underlag för fastställande av hygieniskt gränsvärde. Volymen omfattar de underlag som avgivits från Kriteriegruppen för hygieniska gränsvärden under perioden oktober 2005 - juni 2006.

Nyckelord: Ammoniak, Dikväveoxid, n-Hexanal, Hygieniskt gränsvärde, Lustgas, Penicilliner, Riskvärdering, Toxikologi, Vetenskapligt underlag.

En svensk version av dessa vetenskapliga underlag finns publicerad i Arbete och Hälsa 2006:9.

APPENDIX

Consensus reports in this and previous volumes

Substance	Consensus date	Volume in Arbete och Hälsa	(No.)
Acetaldehyde	February 17, 1987	1987:39	(VIII)
Acetamide	December 11, 1991	1992:47	(XIII)
Acetic acid	June15, 1988	1988:32	(IX)
Acetone	October 20, 1987	1988:32	(IX)
Acetonitrile	September 12, 1989	1991:8	(XI)
Acrylamide	April 17, 1991	1992:6	(XII)
Acrylates	December 9, 1984	1985:32	(VI)
Acrylonitrile	April 28, 1987	1987:39	(VIII)
Aliphatic amines	August 25, 1982	1983:36	(IV)
Aliphatic hydrocarbons, C_{10}-C_{15}	June 1, 1983	1983:36	(IV)
Aliphatic monoketons	September 5, 1990	1992:6	(XII)
Allyl alcohol	September 9, 1986	1987:39	(VIII)
Allylamine	August 25, 1982	1983:36	(IV)
Allyl chloride	June 6, 1989	1989:32	(X)
Aluminum	April 21, 1982	1982:24	(III)
revised	September 14, 1994	1995:19	(XVI)
Aluminum trifluoride	September 15, 2004	2005:17	(XXVI)
p-Aminoazobenzene	February 29, 1980	1981:21	(I)
Ammonia	April 28, 1987	1987:39	(VIII)
revised	October 24, 2005	2006:11	(XXVII)
Ammonium fluoride	September 15, 2004	2005:17	(XXVI)
Amylacetate	March 23, 1983	1983:36	(IV)
revised	June 14, 2000	2000:22	(XXI)
Aniline	October 26, 1988	1989:32	(X)
Anthraquinone	November 26,1987	1988:32	(IX)
Antimony + compounds	December 8, 1999	2000:22	(XXI)
Arsenic, inorganic	December 9, 1980	1982:9	(II)
revised	February 15, 1984	1984:44	(V)
Arsine	October 20, 1987	1988:32	(IX)
Asbestos	October 21, 1981	1982:24	(III)
Barium	June 16, 1987	1987:39	(VIII)
revised	January 26, 1994	1994:30	(XV)
Benzene	March 4, 1981	1982:9	(II)
revised	February 24, 1988	1988:32	(IX)
Benzoyl peroxide	February 13, 1985	1985:32	(VI)
Beryllium	April 25, 1984	1984:44	(V)
Borax	October 6, 1982	1983:36	(IV)
Boric acid	October 6, 1982	1983:36	(IV)
Boron Nitride	January 27 1993	1993:37	(XIV)
Butadiene	October 23, 1985	1986:35	(VII)
1-Butanol	June 17, 1981	1982:24	(III)
Butanols	June 6, 1984	1984:44	(V)
Butyl acetate	June 6, 1984	1984:44	(V)
Butyl acetates	February 11, 1998	1998:25	(XIX)
Butylamine	August 25, 1982	1983:36	(IV)
Butyl glycol	October 6, 1982	1983:36	(IV)
γ-Butyrolactone	June 2 2004	2005:7	(XXV)

Cadmium	January 18, 1980	1981:21	(I)
revised	February 15, 1984	1984:44	(V)
revised	May 13, 1992	1992:47	(XIII)
revised	February 5 2003	2003:16	(XXIV)
Calcium fluorid	September 15, 2004	2005:17	(XXVI)
Calcium hydroxide	February 24, 1999	1999:26	(XX)
Calcium nitride	January 27, 1993	1993:37	(XIV)
Calcium oxide	February 24, 1999	1999:26	(XX)
Caprolactam	October 31, 1989	1991:8	(XI)
Carbon monoxide	December 9, 1981	1982:24	(III)
Cathecol	September 4, 1991	1992:47	(XIII)
Chlorine	December 9, 1980	1982:9	(II)
Chlorine dioxide	December 9, 1980	1982:9	(II)
Chlorobenzene	September 16 1992	1993:37	(XIV)
revised	April 2 2003	2003:16	(XXIV)
o-Chlorobenzylidene malononitrile	June 1, 1994	1994:30	(XV)
Chlorocresol	December 12, 1990	1992:6	(XII)
Chlorodifluoromethane	June 2, 1982	1982: 24	(III)
Chlorophenols	September 4, 1985	1986:35	(VII)
Chloroprene	April 16, 1986	1986:35	(VII)
Chromium	December 14, 1979	1981:21	(I)
revised	May 26, 1993	1993:37	(XIV)
revised	May 24, 2000	2000:22	(XXI)
Chromium trioxide	May 24, 2000	2000:22	(XXI)
Coal dust	September 9, 1986	1987:39	(VIII)
Cobalt	October 27, 1982	1983:36	(IV)
Cobalt and cobalt compounds	October 22 2003	2005:7	(XXV)
Copper	October 21, 1981	1982:24	(III)
Cotton dust	February14, 1986	1986:35	(VII)
Creosote	October 26, 1988	1989:32	(X)
Cresols	February 11, 1998	1998:25	(XIX)
Cumene	June 2, 1982	1982:24	(III)
Cyanamid	September 30, 1998	1999:26	(XX)
Cyanoacrylates	March 5, 1997	1997:25	(XVIII)
Cycloalkanes, C_5-C_{15}	April 25, 1984	1984:44	(V)
Cyclohexanone	March 10, 1982	1982:24	(III)
revised	February 24 1999	1999:26	(XX)
Cyclohexanone peroxide	February 13, 1985	1985:32	(VI)
Cyclohexylamine	February 7, 1990	1991:8	(XI)
Desflurane	May 27, 1998	1998:25	(XIX)
Diacetone alcohol	December 14, 1988	1989:32	(X)
Dichlorobenzenes	February 11, 1998	1998:25	(XIX)
1,2-Dibromo-3-chloropropane	May 30, 1979	1981:21	(I)
Dichlorodifluoromethane	June 2, 1982	1982:24	(III)
1,2-Dichloroethane	February 29, 1980	1981:21	(I)
Dichloromethane	February 29, 1980	1981:21	(I)
Dicumyl peroxide	February 13, 1985	1985:32	(VI)
Dicyclopentadiene	March 23, 1994	1994:30	(XV)
Diesel exhaust	December 4 2002	2003:16	(XXIV)
Diethanolamine	September 4, 1991	1992:47	(XIII)
Diethylamine	August 25, 1982	1983:36	(IV)
2-Diethylaminoethanol	January 25, 1995	1995:19	(XVI)
Diethylene glycol	September 16, 1992	1993:37	(XIV)
Diethyleneglycol ethylether + acetate	December 11, 1996	1997:25	(XVIII)
Diethyleneglycol methylether + acetate	March 13, 1996	1996:25	(XVII)
Diethyleneglycol monobutylether	January 25, 1995	1995:19	(XVI)
Diethylenetriamine	August 25, 1982	1983:36	(IV)

revised	January 25,	1995	1995:19	(XVI)
Diisocyanates	April 8,	1981	1982:9	(II)
revised	April 27,	1988	1988:32	(IX)
Diisopropylamine	February 7,	1990	1991:8	(XI)
N,N-Dimethylacetamide	March 23,	1994	1994:30	(XV)
Dimethyl adipate	December 9,	1998	1999:26	(XX)
Dimethylamine	December 10,	1997	1998:25	(XIX)
N,N-Dimethylaniline	December 12,	1989	1991:8	(XI)
Dimethyldisulfide	September 9,	1986	1987:39	(VIII)
Dimethylether	September 14,	1994	1995:19	(XVI)
Dimethylethylamine	June 12,	1991	1992:6	(XII)
Dimethylformamide	March 23,	1983	1983:36	(IV)
Dimethyl glutarate	December 9,	1998	1999:26	(XX)
Dimethylhydrazine	January 27,	1993	1993:37	(XIV)
Dimethyl succinate	December 9,	1998	1999:26	(XX)
Dimethylsulfide	September 9,	1986	1987:39	(VIII)
Dimethylsulfoxide, DMSO	December 11,	1991	1992:47	(XIII)
Dioxane	August 25,	1982	1983:36	(IV)
revised	March 4,	1992	1992:47	(XIII)
Diphenylamine	January 25,	1995	1995:19	(XVI)
4,4'-Diphenylmethanediisocyanate (MDI)	April 8,	1981	1982:9	(II)
reviderat	May 30	2001	2001:20	(XXII)
Dipropylene glycol	May 26,	1993	1993:37	(XIV)
Dipropyleneglycol monomethylether	December 12,	1990	1992:6	(XII)
Disulfiram	October 31,	1989	1991:8	(XI)
Enzymes, industrial	June 5,	1996	1996:25	(XVII)
Ethanol	May 30,	1990	1991:8	(XI)
Ethanolamine	September 4,	1991	1992:47	(XIII)
Ethylacetate	March 28,	1990	1991:8	(XI)
Ethylamine	August 25,	1982	1983:36	(IV)
Ethylamylketone	September 5,	1990	1992:6	(XII)
Ethylbenzene	December 16,	1986	1987:39	(VIII)
Ethylchloride	December 11,	1991	1992:47	(XIII)
Ethylene	December 11,	1996	1997:25	(XVIII)
Ethylene chloride	February 29,	1980	1981:21	(I)
Ethylene diamine	August 25,	1982	1983:36	(IV)
Ethylene glycol	October 21,	1981	1982:24	(III)
Ethylene glycol methylether + acetate	June 2,	1999	1999:26	(XX)
Ethyleneglycol monoisopropylether	November 16,	1994	1995:19	(XVI)
Ethyleneglycol monopropylether + acetate	September 15,	1993	1994:30	(XV)
Ethylene oxide	December 9,	1981	1982:24	(III)
Ethylenethiourea	September 27,	2000	2001:20	(XXII)
Ethylether	January 27,	1993	1993:37	(XIV)
Ethylglycol	October 6,	1982	1983:36	(IV)
Ferbam	September 12,	1989	1991:8	(XI)
Ferric dimethyldithiocarbamate	September 12,	1989	1991:8	(XI)
Flour dust	December 10,	1997	1998:25	(XIX)
Fluorides	September 15,	2004	2005:17	(XXVI)
Formaldehyde	June 30,	1979	1981:21	(I)
revised	August 25,	1982	1983:36	(IV)
Formamide	December 12,	1989	1991:8	(XI)
Formic acid	June 15,	1988	1988:32	(IX)
Furfural	April 25,	1984	1984:44	(V)
Furfuryl alcohol	February 13,	1985	1985:32	(VI)
Gallium + Gallium compounds	January 25,	1995	1995:19	(XVI)

Glutaraldehyde	September 30	1998	1999:26	(XX)
Glycol ethers	October 6,	1982	1983:36	(IV)
Glyoxal	September 13,	1996	1996:25	(XVII)
Grain dust	December 14,	1988	1989:32	(X)
Graphite	December 10,	1997	1998:25	(XIX)
Halothane	April 25,	1985	1985:32	(VI)
2-Heptanone	September 5,	1990	1992:6	(XII)
3-Heptanone	September 5,	1990	1992:6	(XII)
Hexachloroethane	September 15,	1993	1994:30	(XV)
Hexamethylenediisocyanate (HDI)	April 8,	1981	1982:9	(II)
revised	May 30,	2001	2001:20	(XXII)
Hexamethylenetetramine	August 25,	1982	1983:36	(IV)
n-Hexanal	March 29,	2006	2006:11	(XXVII)
n-Hexane	January 27,	1982	1982:24	(III)
2-Hexanone	September 5,	1990	1992:6	(XII)
Hexyleneglycol	November 17,	1993	1994:30	(XV)
Hydrazine	May 13,	1992	1992:47	(XIII)
Hydrogen bromide	February 11,	1998	1998:25	(XIX)
Hydrogen cyanide	February 7	2001	2001:20	(XXII)
Hydrogen fluoride	April 25,	1984	1984:44	(V)
revised	September 15,	2004	2005:17	(XXVI)
Hydrogen peroxide	April 4,	1989	1989:32	(X)
Hydrogen sulfide	May 4,	1983	1983:36	(IV)
Hydroquinone	October 21,	1989	1991:8	(XI)
Indium	March 23,	1994	1994:30	(XV)
Industrial enzymes	June 5,	1996	1996:25	(XVII)
Isocyanic Acid (ICA)	December 5	2001	2002:19	(XXIII)
Isophorone	February 20,	1991	1992:6	(XII)
Isopropanol	December 9,	1981	1982:24	(III)
Isopropylamine	February 7,	1990	1991:8	(XI)
Isopropylbenzene	June 2,	1982	1982:24	(III)
Lactates	March 29,	1995	1995:19	(XVI)
Lactate esters	June 2,	1999	1999:26	(XX)
Laughing gas	June 7;	2006	2006:11	(XXVII)
Lead, inorganic	February 29,	1980	1981:21	(I)
revised	September 5,	1990	1992:6	(XII)
revised	December 8,	2004	2005:17	(XXVI)
Lithium and lithium compounds	June 4	2003	2003:16	(XXIV)
Lithium boron nitride	January 27,	1993	1993:37	(XIV)
Lithium nitride	January 27,	1993	1993:37	(XIV)
Maleic anhydride	September 12,	1989	1991:8	(XI)
Manganese	February 15,	1983	1983:36	(IV)
revised	April 17,	1991	1992:6	(XII)
revised	June 4,	1997	1997:25	(XVIII)
Man made mineral fibers	March 4,	1981	1982:9	(II)
revised	December 1,	1987	1988:32	(IX)
Mercury, inorganic	April 25,	1984	1984:44	(V)
Mesityl oxide	May 4,	1983	1983:36	(IV)
Metal stearates, some	September 15,	1993	1994:30	(XV)
Methacrylates	September 12,	1984	1985:32	(VI)
Methanol	April 25,	1985	1985:32	(VI)
Methyl acetate	March 28	1990	1991:8	(XI)
Methylamine	August 25,	1982	1983:36	(IV)
Methylamyl alcohol	March 17,	1993	1993:37	(XIV)

Methyl bromide	April 27, 1988	1988:32	(IX)
Methyl chloride	March 4, 1992	1992:47	(XIII)
Methyl chloroform	March 4, 1981	1982:9	(II)
4,4´-methylene-bis-(2-chloroaniline)	February 4 2004	2005:7	(XXV)
Methylene chloride	February 29, 1980	1981:21	(I)
4,4'-Methylene dianiline	June 16, 1987	1987:39	(VIII)
revised	October 3 2001	2002:19	(XXIII)
Methyl ethyl ketone	February 13, 1985	1985:32	(VI)
Methyl ethyl ketone peroxide	February 13, 1985	1985:32	(VI)
Methyl formate	December 12, 1989	1991:8	(XI)
Methyl glycol	October 6, 1982	1983:36	(IV)
Methyl iodide	June 30, 1979	1981:21	(I)
Methylisoamylamine	September 5, 1990	1992:6	(XII)
Methylisoamylketone	February 6 2002	2002:19	(XXIII)
Methylisocyanate (MIC)	December 5 2001	2002:19	(XXIII)
Methyl mercaptane	September 9, 1986	1987:39	(VIII)
Methyl methacrylate	March 17, 1993	1993:37	(XIV)
Methyl pyrrolidone	June 16, 1987	1987:39	(VIII)
α-Methylstyrene	November 1 2000	2001:20	(XXII)
Methyl-t-butyl ether	November 26, 1987	1988:32	(IX)
revised	September 30, 1998	1999:26	(XX)
Mixed solvents, neurotoxicity	April 25, 1985	1985:32	(VI)
MOCA	February 4 2004	2005:7	(XXV)
Molybdenum	October 27, 1982	1983:36	(IV)
Monochloroacetic acid	February 20, 1991	1992:6	(XII)
Monochlorobenzene	September 16,1993	1993:37	(XIV)
Monomethylhydrazine	March 4, 1992	1992:47	(XIII)
Mononitrotoluene	February 20, 1991	1992:6	(XII)
Monoterpenes	February 17, 1987	1987:39	(VIII)
Morpholine	December 8, 1982	1983:36	(IV)
revised	June 5, 1996	1996:25	(XVII)
Naphthalene	May 27, 1998	1998:25	(XIX)
Natural crystallinic fibers (except asbestos)	June 12, 1991	1992:6	(XII)
Nickel	April 21, 1982	1982:24	(III)
Nicotine	June 2 2004	2005:7	(XXV)
Nitroethane	April 4, 1989	1989:32	(X)
Nitrogen oxides	December 11, 1985	1986:35	(VII)
Nitroglycerin	February 13, 1985	1985:32	(VI)
Nitroglycol	February 13, 1985	1985:32	(VI)
Nitromethane	January 6, 1989	1989:32	(X)
Nitropropane	October 28, 1986	1987:39	(VIII)
2-Nitropropane	March 29, 1995	1995:19	(XVI)
Nitroso compounds	December 12, 1990	1992:6	(XII)
Nitrosomorpholine	December 8, 1982	1983:36	(IV)
Nitrotoluene	February 20, 1991	1992:6	(XII)
Nitrous oxide	December 9, 1981	1982:24	(III)
revised	June 7, 2006	2006:11	(XXVII)
Oil mist	April 8, 1981	1982:9	(II)
Organic acid anhydrides, some	September 12, 1989	1991:8	(XI)
Oxalic acid	February 24, 1988	1988:32	(IX)
Ozone	April 28, 1987	1987:39	(VIII)
Paper dust	February 7, 1990	1991:8	(XI)
Penicillins	November 23, 2005	2006:11	(XXVII)
Pentaerythritol	November 16, 1994	1995:19	(XVI)
1,1,1,2,2-Pentafluoroethane	February 24, 1999	1999:26	(XX)

Pentyl acetate	June 14, 2000	2000:22	(XXI)
Peroxides, organic	February 13, 1985	1985:32	(VI)
Phenol	February 13, 1985	1985:32	(VI)
Phosphorous chlorides	September 30, 1998	1999:26	(XX)
Phosphorous oxides	February 11, 1998	1998:25	(XIX)
Phthalates	December 8, 1982	1983:36	(IV)
Phthalic anhydride	September 12, 1989	1991:8	(XI)
Piperazine	September 12, 1984	1985:32	(VI)
Plastic dusts	December 16, 1986	1987:39	(VIII)
Platinum	June 4, 1997	1997:25	(XVIII)
Polyaromatic hydrocarbons	February 15, 1984	1984:44	(V)
Polyisocyanates	April 27, 1988	1988:32	(IX)
Potassium aluminium fluoride	June 4, 1997	1997:25	(XVIII)
Potassium cyanide	February 7 2001	2001:20	(XXII)
Potassium dichromate	May 24, 2000	2000:22	(XXI)
Potassium Fluoride	September 15, 2004	2005:17	(XXVI)
Potassium hydroxide	Marsh 15, 2000	2000:22	(XXI)
2-Propanol	December 9, 1981	1982:24	(III)
Propene	September 13, 1996	1996:25	(XVII)
Propionic acid	November 26, 1987	1988:32	(IX)
Propylacetate	September 14, 1994	1995:19	(XVI)
Propylene glycol	June 6, 1984	1984:44	(V)
Propylene glycol-1,2-dinitrate	May 4, 1983	1983:36	(IV)
Propylene glycol monomethylether	October 28, 1986	1987:39	(VIII)
Propylene oxide	June 11, 1986	1986:35	(VII)
Pyridine	May 13, 1992	1992:47	(XIII)
Quartz	March 13, 1996	1996:25	(XVII)
Resorcinol	September 4, 1991	1992:47	(XIII)
Selenium	December 11, 1985	1986:35	(VII)
revised	February 22, 1993	1993:37	(XIV)
Sevoflurane	May 27, 1998	1998:25	(XIX)
Silica	March 13, 1996	1996:25	(XVII)
Silver	October 28, 1986	1987:39	(VIII)
Sodium cyanide	February 7 2001	2001:20	(XXII)
Sodium Fluoride	September 15, 2004	2005:17	(XXVI)
Sodium hydroxide	August 24, 2000	2000:22	(XXI)
Stearates, metallic, some	September 15, 1993	1994:30	(XV)
Stearates, non-metallic, some	November 17, 1993	1994:30	(XV)
Strontium	January 26, 1994	1994:30	(XV)
Styrene	February 29, 1980	1981:21	(I)
revised	October 31, 1989	1991:8	(XI)
Sulfur dioxide	April 25, 1985	1985:32	(VI)
Sulfur fluorides	March 28, 1990	1991:8	(XI)
Synthetic inorganic fibers	March 4, 1981	1982:9	(II)
revised	December 1, 1987	1988:32	(IX)
revised	December 3, 2003	2005:7	(XXV)
Synthetic organic and inorganic fibers	May 30, 1990	1991:8	(XI)
Talc dust	June 12, 1991	1992:6	(XII)
Terpenes, mono-	February 17, 1987	1987:39	(VIII)
Tetrabromoethane	May 30, 1990	1991:8	(XI)
Tetrachloroethane	June 4, 1997	1997:25	(XVIII)
Tetrachloroethylene	February 29, 1980	1981:21	(I)
1,1,1,2-Tetrafluoroethane	March 29, 1995	1995:19	(XVI)
Tetrahydrofuran	October 31, 1989	1991:8	(XI)

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

Sent for publication December 2006