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The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals

137. Ammonia

Jyrki Liesivuori

Nordic Council of Ministers

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Preface

The Nordic Council of Ministers is an intergovernmental collaborative body for the five countries, Denmark, Finland, Iceland, Norway, and Sweden. One of the committees, the Nordic Senior Executive Committee for Occupational Environmental Matters, initiated a project in order to produce criteria documents to be used by the regulatory authorities in the Nordic countries as a scientific basis for the setting of national occupational exposure limits.

The management of the project is given to an expert group. At present the Nordic Expert Group (NEG) consists of the following members:

Gunnar Johanson, chairman	Karolinska Institutet and National Institute for Working Life, Sweden
Vidir Kristjansson	Administration of Occupational Safety and Health, Iceland
Kai Savolainen	Finnish Institute of Occupational Health, Finland
Vidar Skaug	National Institute of Occupational Health, Norway
Karin Sørig Hougaard	National Institute of Occupational Health, Denmark

For each document an author is appointed by NEG and the national member acts as a referent. The author searches for literature in different data bases such as HSELINE, Medline and NIOSHTIC. Information from other sources such as WHO, NIOSH and the Dutch Expert Committee on Occupational Standards (DECOS) is also used as are handbooks such as Patty's Industrial Hygiene and Toxicology. Evaluation is made of all relevant scientific original literature found. In exceptional cases information from documents difficult to access is used. Whereas NEG adopts the document by consensus procedures, thereby granting the quality and conclusions, the author is responsible for the factual content of the document.

The document aims at establishing dose-response/dose-effect relationships and defining a critical effect based only on the scientific literature. The task is not to give a proposal for a numerical occupational exposure limit value.

The evaluation of the literature and the drafting of this document on ammonia was made by Dr Jyrki Liesivuori, University of Kuopio, and Finnish Institute of Occupational Health, Finland. The final version was accepted by NEG September 9, 2005. Editorial work and technical editing was performed by NEGs scientific secretaries, Anna-Karin Alexandrie and Jill Järnberg, at the National Institute for Working Life in Sweden.

All criteria documents produced by NEG may be downloaded from *www.nordicexpertgroup.org*.

We acknowledge the Nordic Council of Ministers for its financial support of this project.

Anna-Karin Alexandrie, Jill Järnberg
Scientific Secretaries

Gunnar Johanson
Chairman

Abbreviations and acronyms

ACGIH	American Conference of Governmental Industrial Hygienists
ATP	adenosine triphosphate
CA	chromosomal aberration
CI	confidence interval
DEPC	diethyl pyrocarbonate
EPA	United States Environmental Protection Agency
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
FIV	forced inspiratory volume
IPCS	International Programme on Chemical Safety
LC_{50}	lethal concentration for 50% of the exposed animals at single exposure
LD_{50}	lethal dose for 50% of the exposed animals at single administration
LOAEL	lowest observed adverse effect level
MNNG	*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine
MRL	minimal risk level
NIOSH	United States National Institute of Occupational Safety and Health
NMDA	*N*-methyl-D-aspartate
NOAEL	no observed adverse effect level
OSHA	Occupational Safety and Health Association
PEFR	peak expiratory flow rate
RADS	reactive airways dysfunction syndrome
RD_{50}	concentration, which produce a 50% decrease in respiratory rate
SCE	sister chromatid exchange
SPIN	Substances in Preparation in Nordic Countries
STEL	short-term exposure limit
VC	vital capacity

Contents

1. Introduction

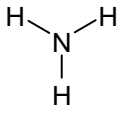
Ammonia (NH_3) is a colourless gas with a distinctly pungent odour at normal atmospheric temperatures and pressures. Ammonia dissolves readily in water and a pH dependent equilibrium is established between NH_3, and ammonium (NH_4^+) and hydroxide (OH^-) ions. The ionised form predominates in water solutions and at physiological pH. NH_3 diffuses more easily than NH_4^+ and can readily pass across membranes. Unless otherwise stated, the term ammonia in this document refers to the sum of NH_3 and NH_4^+.

Ammonia is an endogenous compound produced in different metabolic reactions in the human body. The steady-state level of ammonia in the liver is about 0.7 mM and in blood plasma about ten times lower (151).

The principal source of atmospheric ammonia is animal husbandry and the remainder is largely released from fertilisers (147). A small fraction originates from crops as leaf emissions. Ammonia is one of the most extensively used industrial chemicals. Occupational exposures may occur in ammonia plants, fishing industries, fertiliser manufacturing and animal production (poultry, pigs and fur animals).

This document is limited to anhydrous ammonia and aqueous ammonia solutions although ammonia may also react with other substances to form ammonium compounds including salts such as ammonium chloride, ammonium nitrate, and ammonium sulphate. A criteria document on ammonia was written for the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) in 1986 (110).

2. Substance identification

CAS No.:	7664-41-7
EINECS No.:	2316353
IUPAC name:	ammonia
Molecular formula:	NH_3
Molecular weight:	17.03
Structural formula:	

3. Physical and chemical properties (12, 62, 87)

Boiling point at 101.3 kPa:		-33.4°C
Melting point at 101.3 kPa:		-77.7°C
Vapour pressure at 20°C:		857 kPa
Density, gas at 101.3 kPa and 0°C:		0.7714 g/l
Vapour density (air = 1):		0.6
Density, liquid at 101.3 kPa and -33.4°C:		0.6818 g/l
Viscosity at -33°C:		0.254 centipoise
Viscosity at 20°C:		0.00982 centipoise
Flammability limits:	Lower explosive limit	16% vol
	Upper explosive limit	27% vol
Autoignition temperature:		651°C
Critical temperature:		132.45°C
Critical pressure at 25°C:		11379 kPa
Solubility in water at 101 kPa and:	0°C	895 g/l
	20°C	529 g/l
	40°C	316 g/l
	60°C	168 g/l
Partition coefficients:	Log $K_{ow\ (octanol/water)}$	0.23
	Log $K_{oc\ (octanol/carbon)}$	1.155
Odour threshold:		5-6 ppm
Conversion factors at 25°C:		1 mg/m^3 = 1.4 ppm 1 ppm = 0.7 mg/m^3

Ammonia (NH_3) is a colourless gas with a distinctly pungent odour under normal conditions. It can be compressed and become a liquid under high pressure. NH_3 easily dissolves in water. In aqueous solution, NH_3 acts as a base, acquiring hydrogen ions from water to yield ammonium (NH_4^+) and hydroxide (OH^-) ions. The equilibrium between NH_3 and NH_4^+ is pH-dependent. The pK_a for ammonia depends on the temperature, being 9.1-9.2 at 37°C. Thus, under normal physiological conditions, more that 98% of ammonia is present as NH_4^+ (56).

The given odour threshold is obtained from Devos *et al*, who based their estimates on 11 studies (45). In a more recent study by Sundblad *et al* the subjects detected the smell of ammonia at 5 ppm, the lowest concentration tested (173).

4. Occurrence, production and use

Ammonia is one of the principal compounds in the natural cycle of nitrogen in the environment. It is present in air both as a gas, as ammonium salts in aerosols, and as ammonium ions dissolved in droplets after reactions with acidic air components, e.g. sulphur dioxide. Ammonia concentrations are higher over continents than over oceans and also higher in urban than in rural areas. Reported levels vary from 1 to 16 $\mu g/m^3$ (1.4-23 ppb). The concentrations tend to be higher in summer than in winter (11). Ammonia can be detected in indoor air, as emissions from textiles and some floor and wall materials. Ammonia is present in several agricultural activities and is formed in animal facilities mainly from manure (53).

Ammonia is one of the most commonly produced inorganic compounds in the chemical industry. World industrial ammonia production grew from 119 million tons in 1980 to a peak of 141 million tons in 1989. Since then, the production has remained relatively stable with increases only in Asia for fertiliser production. Use of ammonia in other industrial processes does not seem to increase further (86). For industrial use, ammonia is synthesised from nitrogen and hydrogen with catalysts by the Haber-Bosch method. Eighty-five per cent of the total production is further processed for fertiliser production. Anhydrous ammonia is widely used as an inexpensive fertiliser (169). Ammonia was used as a coolant for decades, but other compounds have now replaced it as freezing agent in industrial refrigerators. However, ammonia is still used in a wide range of commercial and consumer products, including caustic cleaners, chemical synthesis, explosives, plastics, and dyes.

Ammonia gas is commercially available in a number of grades depending on its intended use with a minimum purity of 99.5%. Ammonia is shipped and stored as a liquefied gas under high pressure. It is also available in water solution with the most common commercial formulation containing 28-30% ammonia. Solutions of greater than 25-30% readily give off ammonia gas at normal temperatures. Household products typically contain lower levels of ammonia ranging between 5 and 10% (32).

The use of ammonia in the Nordic countries as reported in the chemical product data base “Substances in Preparation in Nordic Countries” (SPIN), both as totals and for different purposes, is shown in Table 1.

Ammonia is one of the by-products of protein metabolism, and it is normally found in the blood of healthy human subjects at levels below 0.05 mM (41) and in saliva at concentrations of 2.5 mM (84). The steady-state level of ammonia in liver has been reported to be 0.7 mM (151). The concentration of urinary ammonia ranges from 8 to 80 mM in healthy people or approximately 1 000-fold higher than in serum ammonia (95). Muscular activity results in ammonia release and blood and urinary levels may increase after exercise (135). Estimated ammonia production from various substrates in the human intestines ranges from 10 mg/day in the duodenum to 3 080 mg/day in the colon and faecal contents.

Table 1. Annual ammonia consumption in the Nordic countries as reported for 2002 in the chemical product data base "Substances in Preparation in Nordic Countries" (SPIN) (170).

Country	Use category	Tonnes
Finland	Total	374 120
Sweden	Total	209 996
	Raw materials	114 889
	pH-regulating agents	862
	Fertilisers	381
	Galvano-technical agents	344
	Metal-staining agents	162
Norway	Total	160 141
	Raw materials and intermediate products	160 105
	Cleaning and washing agents	29
Denmark	Total	11 311

Nearly all the ammonia produced in the intestines is absorbed (172). Some drugs and chemicals may affect liver and kidney functions and cause an increase of serum ammonia levels. This is seen, for example, after valproate therapy in patients with epilepsy (186) or after exposure to ethylene glycol ethers (103).

5. Measurements and analysis of workplace exposure

The most widely used technique for the sampling of ammonia in workshop air is an impinger flask containing diluted sulphuric acid. Samples are then analysed with a gas selective electrode (137). Another possibility is Nesslerisation in which ammonia reacts in dilute sulphuric or boric acid with an alkaline mercuric and potassium iodide solution to form a brown complex. The concentration is obtained by spectrophotometry, with the absorbance value at 440 nm being compared with a standard (6). A more sophisticated way to analyse ammonia is by ion chromatography (73). Also direct-reading instruments based on infrared spectroscopy have been shown suitable for measuring ammonia in air. Detector tubes specific for ammonia are available for measurements in the range 1-17 000 ppm (119).

Ammonia may be present in air in both the vapour and particulate phase as ammonia gas and as ammonium salts. In order to avoid interactions from ammonium salts and to separate the particulate phase, the use of filter packs or sampling tubes coated with a selective adsorbent is recommended (98). The gaseous ammonia is trapped by acids that act as adsorbents on the coated filter or sampling tube. Examples of methods used for determination of ammonia in air are given in Table 2. The recovery is over 95% for most methods.

Table 2. Sampling and analytical methods for determining ammonia in air. Adapted from ATSDR (12).

Preparation method	Analytical method	Detection limit (μg NH_3/sample)	Ref.
Passive collection using H_2SO_4 in liquid sorbent badge	NIOSH method 6701, ion chromatography, conductivity detection	1	(138)
Air samples (stack emissions) collected through an in-stack filter and then bubbled through H_2SO_4	EPA method 30, ion chromatography	1	(52)
Prefilter may be used. Ammonia trapped on H_2SO_4 - treated silica gel	NIOSH method 6015, colorimetric determination of indophenol by visible light spectrophotometry	0.5	(139)
Prefilter may be used. Ammonia trapped on H_2SO_4 - treated silica gel	NIOSH method 6016, ion chromatography	2	(140)
Chromatomembrane cells pre-extract and preconcentrate sample	Ion chromatography with conductivity detection	6	(54)
Collection in H_2SO_4 - coated activated carbon beads in sampling tube	OSHA method ID-188, ion chromatography	2	(19)
Known volume of air drawn through prefilter and H_2SO_4 - treated silica gel	NIOSH method S347, ammonia-specific electrode	Not reported	(171)

In biological samples like blood, plasma, and serum, ammonia is present mainly as ammonium ion. Therefore, the analysis starts with liberation of ammonia by distillation, aeration, ion-exchange chromatography, microdiffusion, or deproteinisation. Ammonia in urine has been measured by Nesslerisation, enzymatic assays, and chromatographic methods (83). Direct-reading blood ammonia checker based on gas specific potentiometry has been used to assay ammonia in biological samples (119, 155). Some examples of methods used for ammonia determination in biological samples are given in Table 3. Sample detection limits were not reported in any source.

Table 3. Analytical methods for determining ammonia in biological samples. Adapted from ATSDR (12).

Sample matrix	Analytical method	Reference
Urine	Colorimetric (Berthelot reaction)	(176)
Urine	Indophenol reaction	(83)
Urine	Glutamate dehydrogenase based auto-analyser method	(95)
Saliva	Membrane based ammonia-selective electrode	(83)
Serum, plasma, whole blood	Colorimetric assay based on indophenol production	(83)
Serum, plasma, whole blood	Titration	(83)
Serum, plasma, whole blood	Membrane based ammonia-selective electrode	(130)

6. Occupational exposure data

Occupational exposure to ammonia occurs primarily via inhalation. Dermal exposure may also occur due to splashes and spills during handling of aqueous solutions. Only in accidental situations may ammonia be swallowed. Ammonia concentrations have been measured at different work-sites from agriculture (animal production) to process work in factories as well as in blue-line printing shops (Table 4).

Table 4. Occupational exposure levels of ammonia at different work-sites. Most measurements were personal samples.

Work-site	Country	Exposure levels		Reference
		mg/m^3	ppm	
Hairdressing salon	Finland	1.4 – 3.5	2.0 – 5.0	(109)
	The Netherlands	0.02 – 0.43	0.03 – 0.62	(184)
Blue-line printing shop	USA	0.7 – 28	1.0 – 40	(181)
Swine confinement buildings	USA	2.3 – 17.5	3.3 – 25.0	(47)
	United Kingdom	1.0 – 9.2	1.5 – 13.2	(39)
	Finland	0.7 – 23.8	1.0 – 34.0	(116)
	Taiwan	< 3.5	< 5.0	(31)
	The Netherlands	0.1 – 17.3	0.2 – 24.7	(74)
	The Netherlands	0.2 – 2.9	0.3 – 4.2	(152)
Sodium carbonate production	USA	6.4 ± 1.0[a]	9.2 ± 1.4[a]	(81)
Animal facility (mouse rooms)	USA	0.07 – 1.0	0.1 – 1.4	(90)
Poultry house	Iran	23.2 ± 3.6[b]	33.2 ± 5.2[b]	(65)
Cage-laying house	Finland	6.2 – 55.2	8.8 – 78.9	(119)
Cage-laying house	Finland	2.1 – 27.9	3.0 – 39.8	(121)
Floor-laying house	Finland	20.1 – 40.3	28.7 – 57.6	(121)
Barn systems	United Kingdom	7.7	11	(195)
Cage systems	United Kingdom	4.9	7	(195)
Agricultural slurry stores	United Kingdom	0 – 2.7	0 – 3.8	(71)
Municipal sewage plants	Finland	0.007 – 3.48	0.01 – 4.97	(92)
Indoor air (during painting)	Sweden	0.3 – 2.7	0.4 – 3.9	(141)
	Finland	0.0007 – 0.052	0.001 – 0.075	(180)
Effluent treatment plant of pulp mill	USA	0.07 – 20.3	1.0 – 29.0	(68)

[a] mean ± standard error of mean.
[b] mean ± standard deviation.

The International Programme on Chemical Safety (IPCS) presents the highest occupational ammonia exposures at mildew-proofing (122.5 ppm), electroplating (53.9 ppm), galvanising (9.8-86.2 ppm), and chemical mixing (58.8-431.2 ppm) (87). In agricultural settings, the ammonia concentrations seem to be higher in poultry houses (mean 1.6-29.6 ppm, range 1.6-72.9) than in cow houses (mean 0.3-7.7 ppm, range 0.1-29.6), and possibly also in swine houses (4.3-20.8 ppm, range 0.23-59.8) (145).

Ammonia seems to be an indoor air pollutant, although it has not been reliably confirmed whether the measured compound is in fact ammonia or amine degradation products from protein-containing gluing material. Ammonia concentrations in indoor air samples vary from 10 to 110 $\mu g/m^3$ (14-154 ppb) as measured in Finnish residencies (28, 180, 187). In Croatia, in the vicinity of a fertiliser plant, indoor air ammonia concentrations ranged from 32 to 352 $\mu g/m^3$ (45-493 ppb) while ambient air ammonia concentrations were of the same order of magnitude, from 4 to 420 $\mu g/m^3$ (6-590 ppb) (66).

Concentrations may be unpredictably high under accidental circumstances where ammonia gas is released into air (131, 174). Because of the sudden nature of accidents only retrospective estimates of exposure levels are available in the literature. There is one estimate of an exposure level, a report of a fatality at a concentration of approximately 10 000 ppm (134). In this case, a victim was filling a tank wagon with a 25% ammonia solution. Later, it was estimated that the ammonia concentrations may have reached 330 000 ppm, at least sporadically (131).

7. Toxicokinetics

7.1 Uptake

Absorption of ammonia is strongly pH dependent. At higher pH, ammonia is present as a gaseous, relatively lipophilic molecule (NH_3), which readily diffuses through cellular and intracellular membranes. At lower pH, ammonia exists as an ion (NH_4^+), with ionic radius and properties similar to that of the potassium ion (K^+). The NH_4^+ ion, like K^+, can only be transported across membranes by carrier-mediated processes (38). The ionised form predominates (more than 98%) at physiological pH.

The primary site of absorption is the upper respiratory tract. However, in case of aerosols in the air and high humidity, ammonia can adsorb onto the aerosols and be carried deeper into the lungs. Ammonia is probably absorbed percutaneously, if high concentrations are spilt on intact skin and have caused skin injury (62). Absorption through the eye has been reported. Ammonia diffused within seconds into cornea, lens, drainage system, and retina. However, the amounts absorbed were not quantified, and absorption into systemic circulation was not investigated (16, 89 cited in 12).

The knowledge of the toxicokinetics of ammonia is limited and information is primarily available from older human experimental studies. When seven volunteers were exposed to an ammonia concentration of 350 mg/m^3 (500 ppm) for 30 minutes the retention was around 75%. The ammonia retention decreased progressively with time, reaching 23% at steady-state. No effect on blood-nitrogen was seen (166). In a study by Landahl and Herman two male volunteers were exposed to ammonia concentrations ranging from 40 to 350 mg/m^3 (57-500 ppm) for a maximum of 2 minutes. About 92% retention was reported and the exposure level did not affect the retention (104). It is estimated in the IPCS document that exposure to 25 ppm ammonia would raise the blood ammonia concentration by only 10% over fasting levels assuming 30% retention (87). This slight increase is evaluated to be well within the normal human capacity to handle ammonia and is unlikely to cause any harm (174).

In healthy subjects, absorbed ammonia is rapidly catabolised by the liver, mainly to urea. Only relatively small amounts reach the systemic circulation from the gastrointestinal tract as a consequence of this first pass effect (172).

Ammonia absorbed from the intestinal tract arises primarily from bacterial degradation of amino and nucleic acids in ingested food, endogenous epithelial debris, and mucosal cell luminal secretions, or from the hydrolysis of urea diffusing from the systemic circulation into the intestinal tract. Ammonia uptake from the human colon, the major site of ammonia production, increases with increasing pH of the luminal contents. An increase in pH raised the proportion of non-ionised ammonia, showing that most of the ammonia transport relies on passive diffusion. However, ammonia transport, although greatly diminished, still occurred when the luminal pH was reduced to 5, suggesting an active transport mechanism for the ammonium ion (29).

In a study of cerebral uptake of labelled ammonia in Rhesus monkeys it was found that after passage of the bolus, a fraction of 40% remained in the brain (148). The brain content of tracer remained almost stable with a half-time of 45 minutes. Similar observations were reported in man albeit with a half-time of 2.3 hours (149).

In rats exposed to ammonia via inhalation 6 hours/day for 5 days, venous blood ammonia increased linearly from a baseline value of 35 ± 18 mM (mean ± standard deviation) to 44 ± 18 mM at 25 ppm and to 105 ± 14 mM at 300 ppm. However, in rats exposed to the same concentrations for 10 or 15 days the relationship between dose and blood ammonia level was lost, suggesting metabolic adaptation (120). Rats were continuously exposed to ammonia concentrations of 15, 32, 310, or 1 157 ppm for 24 hours and blood ammonia concentration was measured 0, 8, 12 and 24 hours after exposure began. The blood ammonia level increased significantly in a linear fashion with increasing exposure after 8 hours of exposure. The levels declined over time, indicating an increased ammonia metabolism (160).

7.2 Distribution

The distribution of ammonia between body compartments is strongly influenced by pH. The non-ionised ammonia is freely diffusible, whereas the ammonium ion is less diffusible and relatively confined in compartments (62). Ammonium ions compete with potassium ions for inward transport over the cytoplasmic membrane, via potassium transport proteins like the Na^+/K^+-ATPase and the $Na^+K^+2Cl^-$-cotransporter (124).

Ammonia enters the brain from blood by diffusion rather than via a saturable transport system. It has been estimated that up to 25% of ammonia may enter the brain as ammonium ion at physiological pH values. The blood transit time through brain is in the order of seconds, and the NH_4^+ to NH_3 conversion rate is too rapid to limit the rate at which ammonia enters the brain. The lower permeability of the blood-brain barrier to NH_4^+ implies that transfer of ammonia is dependent upon arterial blood pH and systemic alkalosis exacerbates ammonia toxicity (146, 192). This is consistent with a higher rate of diffusion of NH_3 into brain at higher blood pH values (56).

Since diffusion of ammonia into the brain is pH dependent, the pH gradient between blood and brain may affect brain ammonia concentrations (56). Assuming a blood pH of 7.4 and a brain intracellular pH of 7.1 under normal physiological conditions, the Hendersson-Hasselbach equation predicts a ratio of brain to blood ammonia concentrations of 2 (38). Experimental ratios range from 1.5 to 3.0. In hyperammonaemia, the ratio may rise to even 8 (27, 56). In chronic liver failure, prior to the onset of encephalopathy, blood ammonia concentrations are increased by three-fold in both experimental animals and humans (115) and brain concentrations are in the 0.3-0.5 mM range (64). The steady-state level of ammonia in the liver of healthy subjects is about 0.7 mM and about ten times lower in blood plasma (151).

7.3 Endogenous ammonia

In humans and several other species ammonia plays a central role in nitrogen metabolism (Figure 1).

Ammonia is both a product of protein and nucleic acid catabolism, and a precursor for non-essential amino acids and certain other nitrogenous compounds. The liver is the major site of ammonia metabolism.

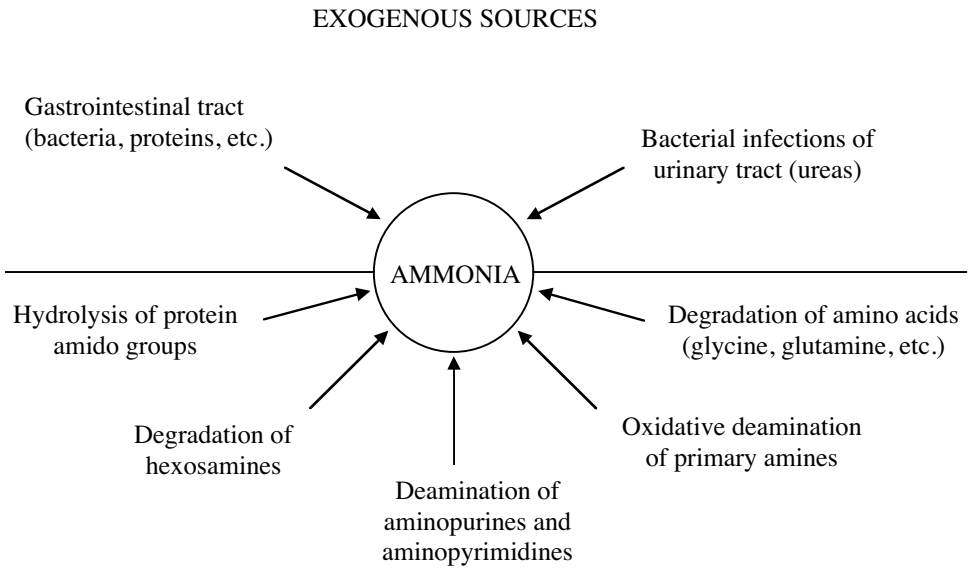


Figure 1. Exogenous and endogenous sources of ammonia in vertebrates. Adapted from Seiler (161).

The major features of nitrogen in the body include:
- release of nitrogen from amino acids, nucleic acids, and amines,
- deamination of glutamate through the action of glutamate dehydrogenase,
- conversion of ammonia to urea by the Krebs-Henseleit (urea) cycle (Figure 2),
- conversion of urea to ammonia in the gastrointestinal tract by the action of bacterial urease, and
- synthesis of glutamine serving as a short-term storage and transport form of ammonia in the glutamine cycle (Figure 3).

The Krebs-Henseleit (urea) cycle is tightly controlled to dispose approximately 90% of the surplus nitrogen (Figure 2).

Glutamine is quantitatively the second major product of hepatic ammonia metabolism and serves an additional important function in the storage and transport of ammonia (Figure 3). The synthesis of glutamine also provides a detoxification mechanism for ammonia in the brain through a single enzymatic step, i.e. via the glutamine synthetase catalysed reaction localised in astrocytes (38). The two nitrogens of glutamine are the major ammonia precursors for ammoniagenesis in the kidney tubular cells of most vertebrates. The ammonia formed in this way enters urine and forms ammonium ions there (151).

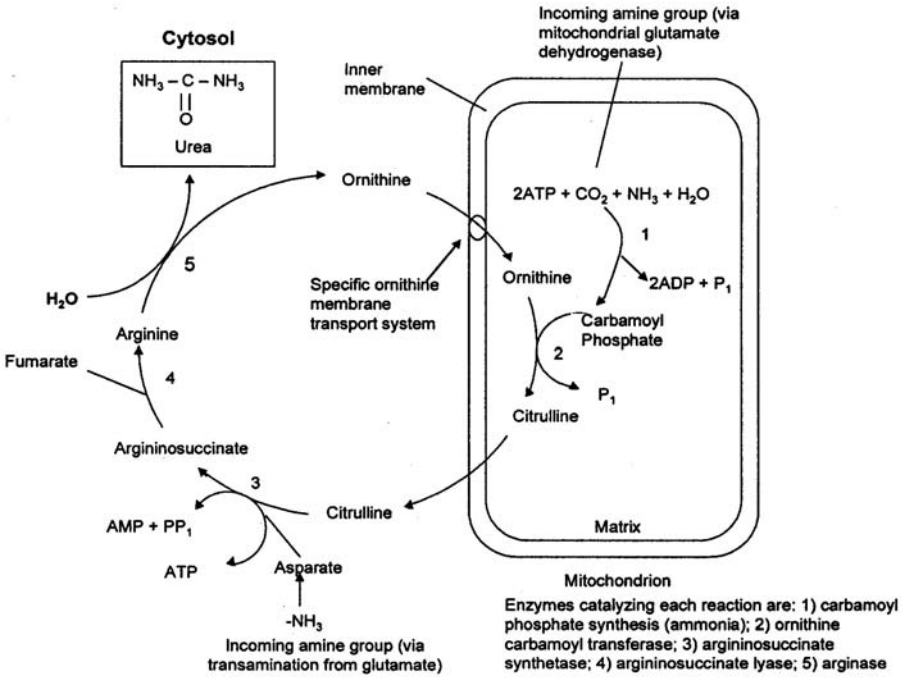


Figure 2. The Krebs-Henseleit (urea) cycle (108). AMP: adenosine monophophate, ADP: adenosine diphoshate, ATP: adenosine triphosphate, P_i: inorganic phosphate (HPO_4^{2-}), and PP_i: pyrophosphate ($P_2O_7^{4-}$).

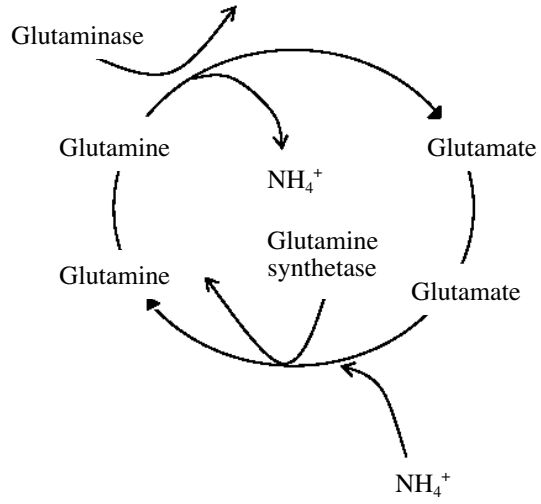


Figure 3. Glutamine cycle. Modified from Brunner and Thaler (25).

Table 5. Arterial blood ammonia concentrations in healthy volunteers and in patients with liver disease (144).

Subjects	Arterial ammonia concentration (mM)	Reference
Healthy volunteers	0.045	(33)
Patients with chronic liver failure and proven cirrhosis	0.060	(33)
Cirrhotic patients with transjugular intrahepatic portosystemic stent shunt[a]	0.080	(156)
Patients with acute-on-chronic liver disease	0.090-0.120	(33)
Patients with acute liver failure	0.150-0.180	(33)
Patients with end stage acute liver failure	0.340	(88)

[a]A non-surgical technique for treatment of refractory ascites associated with cirrhosis of the liver.

Mutch and Bannister reviewed the relationship between muscle activity and ammonia production. The immediate source of ammonia from muscle appears to be a result of the deamination of adenosine monophosphate, which is more apparent in fast twitch fibres than in slow twitch fibres. An increase of blood ammonia levels both in rats after swimming and in humans after manual work, maximal cycle ergometry, and treadmill exercise is observed (135). In a more recent study, heat stress and exercise increased plasma ammonia from preexercise level to 0.06-0.07 mM at the end of submaximal runs, and further to 0.11 mM at the end of performance runs in hot conditions (123). The accumulation of plasma ammonia following sprint exercise is about 35% lower in women than in men (55).

Arterial blood ammonia concentrations in healthy volunteers and in patients with liver disease are shown in Table 5.

A variety of xenobiotics (methanol, formic acid, cyanide, 2-ethyl-hexanoic acid, valproate, and acetaminophen) may impair liver function resulting in increased blood and urine ammonia level (72, 112, 113, 122, 153). Elevated ammonia levels may also be a result from xenobiotics affecting the kidney function. This is seen, for example, after valproate therapy in patients with epilepsy (186) or after exposure to ethylene glycol ethers (103).

Congenital deficiency of enzymes in the urea cycle, such as carbamoyl phosphate synthetase I and, to a lesser extent, ornithine transcarbamylase, as well as several other metabolic disorders like arginosuccinic aciduria may lead to hyperammonaemia and various abnormal urinary constituents (10, 82, 162).

7.4 Biotransformation

Exogenous ammonia, administered intravenously as an ammonium compound, is metabolised to glutamine as the major early product (51). Following administration of ^{13}N-ammonia to rats (via either the carotid artery or cerebrospinal fluid), most of the metabolised label was recovered in glutamine (amide) and minor fractions in glutamate and aspartate. Although ammonia in blood and

cerebrospinal fluid are converted largely to glutamine, it is not possible to predict with certainty the metabolic fate of the bulk of endogenously produced ammonia (36). The ammonia fixed in glutamine may eventually end up in amino acids, purines, pyrimidines, or other nitrogen-containing compounds.

Ingested ammonium chloride and endogenous intestinal ammonia enters the liver via the portal vein and is converted to urea (59, 67, 150). In humans, absorbed ammonia (both exogenous and endogenous) is converted to the ammonium ion as hydroxide and as salts, especially as carbonates. The ammonium salts are then rapidly converted to urea. Protein deamination in the body yields ammonium ions, which are rapidly converted into urea in the liver and excreted by the kidney or used for synthesis of amino acids. Ammonium ions are produced in the kidney in order to maintain electrolyte balance (62).

The hepatic first-pass metabolism of ammonia has been quantified with positron emission tomography (96). The estimate of the hepatic extraction of ammonia in the intact pig was around 0.7. Assuming that 75% of the liver blood flow passes through the portal vein and 25% through the hepatic artery, and using the published values of the blood concentrations in arteries, the hepatic vein, and the portal vein, recalculated values of the hepatic extraction fraction of ammonia were 0.60 in liver patients undergoing surgery, 0.85 in rats and 0.95 in dogs (30, 37, 127). Around one-half of the ammonia is converted to urea in the periportal zone and around one-half to glutamine in the perivenous zone (Figure 4). Urea synthesis accounts for two-thirds of the ammonia utilisation by isolated rat hepatocytes (96).

The synthesis of urea (mechanism for conjugating ammonia into non-toxic compounds for excretion in mammals) requires the concerted action of several enzymes of the urea cycle (Figure 2). One of these, the cytosolic glutamine synthetase, is present in several organs including the brain and the liver.

Glutamine synthesis is the most important alternative pathway for ammonia detoxification. Glutamine synthetase catalyses the synthesis of glutamine from equimolar amounts of glutamate and ammonia (Figure 3) (197). Glutamine is the most abundant free amino acid in the body, with the highest plasma concentration (102). Other organs can take up glutamine where it is split by the intramitochondrial phosphate-dependent enzyme glutaminase into glutamate and ammonia. The glutaminase present in the liver is activated by ammonia, in contrast to other types of glutaminases, which are inhibited by ammonia (40). A schematic representation of ammonia and glutamine trafficking between different organs is given in Figure 4.

Glutamate can be used in transamination reactions, yielding predominantly alanine. Alanine can be released into the bloodstream, and transported to the liver, where the carbon skeleton can be used for gluconeogenesis (144).

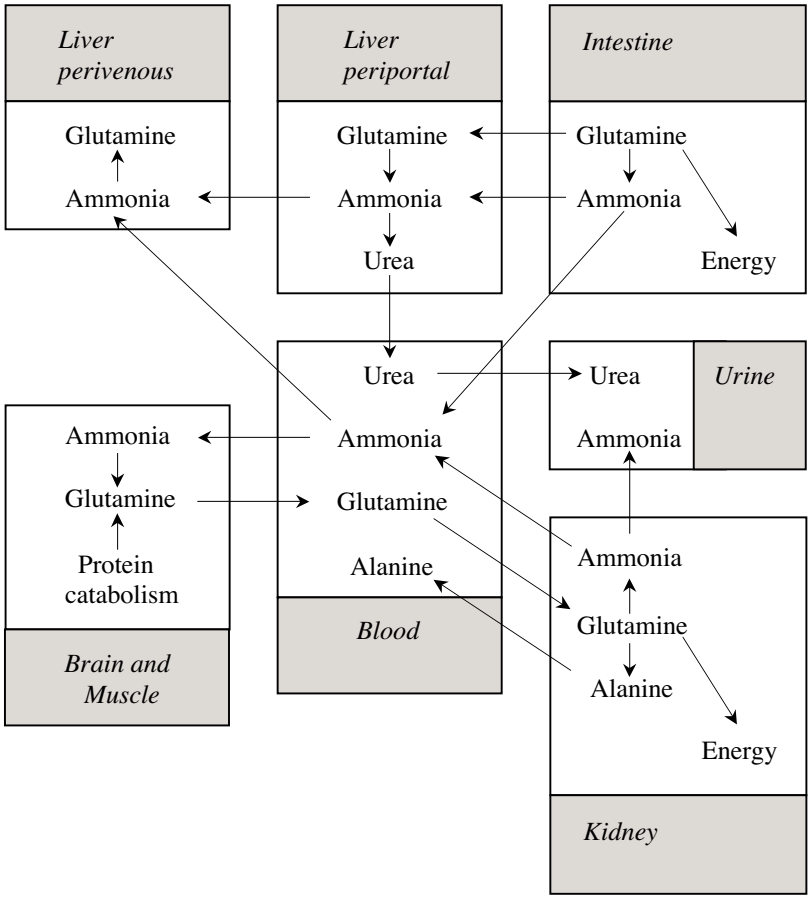


Figure 4. Schematic representation of the predominating ammonia and glutamine turnover between body compartments. Modified from Olde Damink *et al* (144).

7.5 Excretion

In humans, ammonia is primarily excreted via the kidneys. In addition, a significant amount is excreted via the sweat glands (62). Ammonia is mainly excreted as urea by mammals. However, ammonia may also be directly excreted in urine. Glutaminase catalyses the release of ammonia in the kidney tubular epithelium. In acidosis, the renal concentration of glutaminase increases over several days, in parallel with increased excretion of ammonium ions. Two-thirds of the urinary ammonia is excreted via this pathway and approximately one-third is consumed by protein metabolism and ammonia clearance from the plasma by the kidney (91). Renal ammoniagenesis in the proximal tubule is highly increased by chronic metabolic acidosis where glutamine is the major substrate. The mitochondrial glutaminase produces ammonium and glutamate ions. Ammonium is secreted as ammonia and hydrogen ion by separate mechanisms producing ammonium cations in the lumen, and thereby regulating the acid-base balance of the organism (164).

Ammonia may also be excreted through expired air. Reported levels of ammonia in expired air are 0.1-2.2 mg/m^3 (0.15-3.1 ppm) (85) and 0.2-1.2 mg/m^3 (0.3-1.7 ppm) (105). These values, higher than those expected from equilibrium with plasma- and lung-parenchyma- ammonia levels (0.03-0.05 mg/m^3) (0.04-0.07 ppm), are most likely due to the synthesis of ammonia from salivary urea by the oral microflora (62).

Less than 1% of the total ammonia produced in the human intestinal tract (4 g/day) is excreted in the faeces (172).

8. Biological monitoring

Ammonia is endogenously produced and is present in all body fluids. The generally accepted reference level used in clinical laboratories for blood ammonia of healthy subjects is 0.05 mM although lower levels of 0.005-0.007 mM have also been suggested (41). In unexposed healthy subjects the urinary ammonia level is reported to be below 1.4 mol/mol creatinine corresponding to about 10 mM (112). In clinical chemistry, urinary ammonia levels from 8 to 80 mM are regarded as normal values (95). The mean ammonia concentration in saliva of healthy subjects is 2.5 mM (84).

Rats exposed to ammonia vapour showed dose-dependent blood ammonia levels after 5 days of exposure (6 hours per day). However, blood ammonia concentrations had returned to baseline levels after 10 and 15 days of continued exposure (120). In rats continuously exposed to ammonia for 24 hours the level in blood declined over time indicating increased ammonia metabolism during the exposure (160).

There are no reports of increased urinary ammonia excretion after occupational exposure. Inhalation by volunteers of 500 ppm ammonia for 30 minutes did not have any effect on blood and urine nitrogen levels (166). It has been estimated that exposure to 25 ppm ammonia increases the blood ammonia concentration by only 10% over fasting levels, assuming 30% retention (87). However, ammonium concentrations in blood and urine may be assayed for clinical changes caused by ammonium salts and other chemicals affecting the urea cycle like acetaminophen, valproate, 2-ethylhexanoic acid, and formic acid (72, 79, 101, 113).

Due to relatively high endogenous production and adaptive metabolism, bio-monitoring of occupational exposure to ammonia seems of little value.

9. Mechanisms of toxicity

Ammonia as a gas, in anhydrous form or in concentrated solutions, possesses corrosive properties, and massive exposure leads to necrosis of skin and mucous membranes. Ammonia may also cause sensory airway irritation as the trigeminal nerve is affected (131, 174).

The topical damages caused by ammonia are mainly due to its alkaline properties. Because of the high water solubility ammonia dissolves in moisture on the mucous membranes, skin, and eyes, forming ammonium hydroxide, which cause a liquefaction necrosis of the tissues (89). Ammonium hydroxide increases saponification of cell membrane lipids resulting in cell disruption and death. Further, it breaks down cell structural proteins, extracts water from the cells, and initiates an inflammatory response, which damages the adjoining tissues. This reaction is exothermic contributing to tissue damage by cryogenic (thermal) injury in addition to the alkali burns (7, 9).

The most serious effect of elevated endogenous ammonia is seen in the brain as hepatic encephalopathy due to acute or chronic liver failure. Ammonia is metabolised by glutamine synthase localised in astrocytes. Astrocytes are also involved in the uptake of glutamate, an endogenous and most abundant cerebral neurotransmitter that is the primary agonist for *N*-methyl-D-aspartate (NMDA) receptors (142). Thus, the NMDA receptor is also a primary target of ammonia toxicity. The ammonium ion is transported into the cell via the binding sites for K^+ because of similar ionic radius of hydrated ammonium and K^+ (124).

Ammonium neurotoxicity is thus mediated through the activation of NMDA receptors, increased activity of constitutive neuronal nitric oxide synthase, and subsequently increased formation of nitric oxide that serves as a neurotransmitter in a number of neuronal cells (56, 125). Nitric oxide in turn activates guanylate cyclase leading to increased formation of cyclic guanosine monophosphate (77). A key effect of ammonia at the cellular level is oxidative stress due to the generation of reactive oxygen and nitrogen species subsequent to activation of NMDA receptors. These events are associated with mitochondrial dysfunction characterised by increased permeability of mitochondrial transition pore. The opening of the transition pore is associated with cytochrome C release and activation of a cascade of serinine-threonine proteases, also called caspases. Simultaneous decrease of protein kinase C activity is associated with decreased phosphorylation and activity, of Na^+/K^+-ATPase, depletion of ATP (99), and increased levels of free intracellular calcium, toxic to neuronal cells (100). These events are key-elements in the pathway leading to ammonia-induced programmed cell death, apoptosis (143). Key-events of ammonia-induced effects on neuronal cells are depicted in Figure 5.

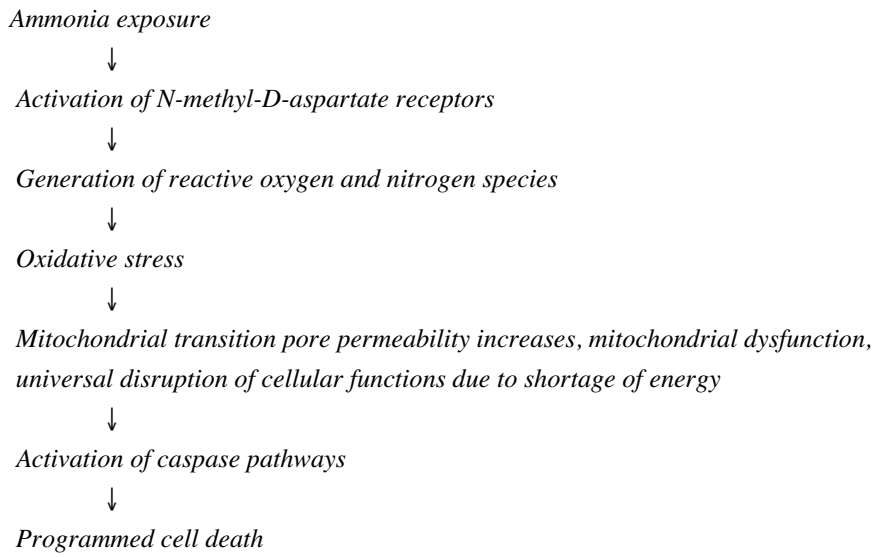


Figure 5. Schematic representation of mechanisms of ammonia-induced neurotoxicity.

10. Effects in animals and *in vitro* studies

10.1 Irritation and sensitisation

Male Swiss-Webster mice were exposed for 30 minutes by inhalation to concentrations of ammonia ranging from 100 to 800 ppm. The maximum depression in respiratory rate at each exposure level occurred within the first 2 minutes with a concentration-effect relationship. The calculated concentration associated with a 50% decrease in respiratory rate (RD_{50}) was 303 ppm (15). In another study where Swiss OF_1 mice were exposed to ammonia the calculated RD_{50} value was 257 ppm. The minimal concentration at which nasal histopathological changes were observed was 711 ppm after a 4-day exposure, 6 hours/day (201).

The National Research Council studied ocular toxicity of ammonia in rabbits (136). Conjunctival oedema with ischaemia and segmentation of limbal vessels were seen after 30 minutes exposure. By 24 hours, there was a reduction in mucopolysaccharide contents of the corneal stroma, and extensive polymorphonuclear infiltration and anterior lens opacities were apparent. In rabbits with corneal burns, neovascularisation occurred after one week, but it was delayed in animals with corneal limbal burns. Complications of severe burns included symblepharon (adhesion of the conjunctival surface between the eyelid and the eyeball), pannus (abnormal membrane-like vascularisation of the cornea), pseudopterygia (a patch of thickened conjunctiva extending over a part of the cornea), progressive or recurrent corneal ulcerations leading to perforations, permanent corneal opacity, corneal staphyloma (a defect in the eye inside the cornea), persistent iritis, phthisis bulbi, secondary glaucoma, and dry eye (136, cited in 87).

No data on sensitisation of animals after ammonia exposure are available.

10.2 Effects of single exposure

In an acute inhalation study on male ICR mice by Kapeghian *et al*, the lethal concentration for 50% of the exposed animals (LC_{50}) at a single 1-hour exposure with a 14-days observation period was calculated to be 2 960 mg/m^3 (4 230 ppm) (93). Lungs of mice that died during the exposure (or during the 14 days observation period) were diffusely haemorrhagic. Histology revealed acute vascular congestion and diffuse intra-alveolar haemorrhage. A mild to moderate degree of chronic focal pneumonitis was also seen. There was evidence of swelling and increased cytoplasmic granularity of hepatocytes and scattered foci of frank cellular necrosis. The acute LC_{50} in male and female Wistar rats was 31 612 mg/m^3 (40 300 ppm) for a 10-minutes exposure and 11 620 mg/m^3 (16 600 ppm) for a 60-minutes exposure (8). During the exposure, clinical signs of restlessness, eye irritation, nasal discharge, mouth breathing, and laboured respiration were seen. Gross necropsy revealed haemorrhagic lungs in animals that died during the study as well as in survivors. In another study, the calculated 1-hour LC_{50} values in rat and mouse of 5 137 and 3 386 mg/m^3 (7 338 and 4 837 ppm), respectively (118) were of the same order as those calculated by Kapeghian *et al* (93). Additional reported LC_{50} values in animals exposed to ammonia are given in Table 6. The cited studies on acute toxicity are somewhat old and were not carried out according to present standards. Nevertheless, taken together they present a consistent picture of short-term LC_{50} values of several thousand ppm.

White rats exposed to ammonia concentrations from 300 to 3 000 mg/m^3 (431-4 307 ppm) for 5-60 minutes expressed decreased static muscle tension, leukocytosis, prolongation of latent reflex time, and increases in total protein, blood sugar, oxygen consumption and residual nitrogen. No changes were observed in rats exposed at 100 mg/m^3 (143 ppm) for 5-60 minutes. A no observed adverse effect level (NOAEL) of 100 mg/m^3 (143 ppm) was concluded from this study (154). Alpatov and Mikhailov regarded 85 mg/m^3 (121 ppm) as a threshold level for acute effects (depression followed by hyperactivity and convulsion) after 120 minutes exposure of albino rats (5, cited in 87).

Clinical and histological effects have also been seen in the lungs of other animal species (cats, mice, and rabbits) following exposure to ammonia gas (46, 60, 167). Cats exposed to 1 000 ppm ammonia gas for 10 minutes and observed for up to 35 days showed a biphasic course of respiratory pathology (46). Effects seen at 24 hours after exposure included severe dyspnoea, anorexia, and dehydration, with rhonchi and course rales evident upon auscultation. Microscopy of lung samples on day 1 showed necrotising bronchitis in the large conducting airways, and necrosis and sloughing of the epithelium, and acute inflammatory reaction in the bronchi. On day 7, the mucosal lesions had resolved, but on day 35, varying degrees of bronchitis and early bronchopneumonia with areas of bulbous emphysema were seen. Gross pathology revealed varying degrees of congestion, haemorrhage, oedema, interstitial emphysema, and collapse of the lungs at all time points. Pulmonary resistance was increased throughout the study (46).

Table 6. Reported LC_{50} values in animals exposed via inhalation.

Species	Exposure duration	LC_{50}		Reference
		mg/m^3	ppm	
White rat	5 min	18 693	26 704	(154)
Mouse	10 min	7 060	10 152	(165)
Wistar rat	10 min	31 612	40 300	(8)
White rat	15 min	12 160	17 372	(154)
White rat	30 min	7 035	10 050	(154)
ICR mouse	1 h	2 960	4 230	(93)
CF_1 mouse	1 h	3 386	4 837	(118)
CFE rat	1 h	5 137	7 338	(118)
White rat	1 h	7 939	11 342	(154)
Wistar rat	1 h	11 620	16 600	(8)
White rat	2 h	7 600	10 860	(4)

LC_{50}: lethal concentration for 50% of the exposed animals at single exposure.

Anaesthetised, mechanically ventilated rabbits exposed to very high levels of nebulised ammonia (2 ml of 23-27% ammonia solution; estimated by the study authors as peak ammonia concentrations of 35 000-39 000 ppm) for 4 minutes had a decrease in blood oxygen saturation and an increase in airway pressure (a measure of changes in airway resistance) (167). Arterial oxygen tension decreased from 23.3 ± 3.6 kPa (mean ± standard deviation) to 11.0 ± 3.6 kPa and peak airway pressure increased from 13 ± 2 cm H_2O (mean ± standard deviation) to 17 ± 2 cm H_2O.

Cardiovascular changes have been observed in rabbits exposed to high concentrations of ammonia for 1 hour (159). Bradycardia was seen at 2 500 ppm, and hypertension and cardiac arrhythmias leading to cardiovascular collapse followed acute exposures to concentrations exceeding 5 000 ppm. Atrophy of pericardial fat has been observed in mice exposed to 4 000 ppm ammonia for 60 minutes (93).

In the LC_{50} study reported in Table 7, male CFE rats and male CF_1 mice were exposed to different ammonia concentrations for 60 minutes. Immediate nasal and eye irritation was followed by laboured breathing and gasping in all study groups. In addition, mild changes in the liver were seen at necropsy (118).

Table 7. Dose-effect relationships for 1-hour inhalation exposure in experimental animals (118).

Species	Exposure level		Effect
	mg/m^3	ppm	
CFE rat	6 888	9 840	Liver fatty infiltration in 1/10 survivors
	5 137	7 338	1-hour LC_{50}
	4 347	6 210	No pathological lesions in 10/10 survivors
CF_1 mouse	4 004	5 720	Mild congestion in the liver in 1/10 survivors
	3 386	4 837	1-hour LC_{50}
	2 520	3 600	No pathological lesions in 10/10 survivors

LC_{50}: lethal concentration for 50% of the exposed animals at single exposure.

10.3 Effects of short-term exposure

Studies in animals have demonstrated both dose-effect and duration-effect relationships in changes at the respiratory tract. Acute exposures to lower ammonia concentrations (less than 1 000 ppm) from 1 hour to 1 week cause airway irritation, whereas exposures to high concentrations (4 000 ppm) for 3 hours to 2 weeks result in severe damage to the upper and lower respiratory tract and alveolar capillaries (35, 93, 126, 158, 160).

Histopathological changes of the respiratory tract were evaluated in rats continuously exposed to a mean ammonia concentration of 200 ppm, range 150-250 ppm, for 12 days. Progressive loss of cilia from and stratification of the tracheal epithelial lining was observed. By day 12 a mucilaginous exudate was apparent in the trachea together with a slight increase in submucosal cellularity (61).

In a 2-month inhalation study on white rats a lowest observed adverse effect level (LOAEL) of 100 mg/m^3 (143 ppm) was determined based on histological changes in the lungs, including small areas of interstitial pneumonia with signs of peribronchitis and perivasculitis. No changes were reported in other organs as compared with the control group. A threshold level for toxic effects of 40 mg/m^3 (57 ppm) was reported from this study (5, cited in 87).

When rats, rabbits, guinea pigs, dogs and monkeys were continuously exposed to ammonia at a concentration of 40 mg/m^3 (57 ppm) for 114 days, no signs of toxicity were seen and gross and microscopic examination did not reveal lung abnormalities (35). A NOAEL of 40 mg/m^3 (57 ppm) can be concluded from this study.

Coon *et al* exposed Sprague-Dawley rats continuously by inhalation to 127 mg/m^3 (181 ppm) and 262 mg/m^3 (374 ppm) for 90 days (48 and 49 animals per group, respectively) and to 455 mg/m^3 (650 ppm) for 65 days (51 animals) (35). The 181 ppm ammonia exposure (NOAEL) did not induce changes in gross or microscopic pathology, haematology, or liver histochemistry. The exposure at 374 ppm (LOAEL) was without specific effects, but mild nasal discharge was seen in 25% of 49 rats. All the 51 rats exposed at 650 ppm showed mild dyspnoea and nasal irritation. There were 32 deaths by day 25, and 50 deaths by day 65 in the 650 ppm group. Myocardial fibrosis was seen in rats, guinea pigs, rabbits, dogs, and monkeys after prolonged (90 days) continuous exposure to 470 mg/m^3 (671 ppm) (35). The contribution of these lesions to the morbidity and mortality of affected animals was not determined.

Broderson *et al* exposed Sherman and Fisher rats to ammonia from natural sources, at an average concentration of 150 ppm for 75 days, and to purified ammonia at 250 ppm for 35 days (23). Histological changes in the olfactory and respiratory epithelia of the nasal cavity were similar in all the exposed rats, showing increased thickness, pyknotic nuclei, and hyperplasia. The submucosa was oedematous with marked dilation of small vessels.

A concentration of 500 ppm was selected in a study of effects of continuous exposure to ammonia, after noting that general toxic effects (particularly on

growth rate) were not present at 250-300 ppm (158). Young male specific-pathogen-free rats were age- and weight-matched with controls (27 animals per group) and exposed for up to 8 weeks. Nasal irritation began on the 4th day. After 3 weeks, exposed rats showed nasal irritation and inflammation of the upper respiratory tract, but no effects were observed on the bronchioles and alveoli. The number of pulmonary alveolar macrophages was similar to that of controls. After 8 weeks, no inflammatory lesions were present.

Swiss mice exposed to 909 ppm ammonia 6 hours/day, 5 days/week for 4-14 days expressed histological lesions in the respiratory epithelium in the nasal cavity. These lesions were not seen at 303 ppm. No lesions were observed in the trachea or lungs at any exposure level (201).

The nasal mucosa was adversely affected in adult male mice exposed to vapours of a 12% ammonia solution for 15 minutes/day, 6 days/week for 4, 5, 6, 7, or 8 weeks (60). Histological changes progressed from weeks 4 to 8 from crowding of cells forming crypts and irregular arrangements to epithelial hyperplasia, patches of squamous metaplasia, loss of cilia, and dysplasia of the nasal epithelium. Carcinomas were seen in two animals (see chapter 10.5 for more details).

Animal studies have revealed that ammonia affects the immune system. Exposure of mice to ammonia at a concentration of 500 ppm for one week followed by exposure to *Pasteurella multocida* at the lethal dose for 50% of the exposed animals (LD_{50}) increased the mortality significantly (158). A significant increase in the severity of respiratory signs characteristic of murine respiratory mycoplasmosis was observed in rats exposed to ammonia at 25 ppm for 4-6 weeks following inoculation with *Mycoplasma pulmonis* intranasally (23). Guinea pigs exposed to 90 ppm for 3 weeks developed a significant decrease in the cell-mediated immune response when challenged with a derivative of tuberculin (175).

Twelve guinea pigs were exposed to an ammonia concentration of about 170 ppm, range 140-200 for 6 hours/day, 5 days a week, for up to 18 weeks. There were no significant findings at autopsy of animals sacrificed after 6 or 12 weeks of exposure. In animals sacrificed after 18 weeks of exposure, there was congestion of the liver, spleen, and kidneys, with early degenerative changes in the adrenal glands. Increased erythrocyte destruction was explained by increased quantities of haemosiderin in the spleen. In the proximal tubules of the kidneys, there was cloudy swelling of the epithelium and precipitated albumin in the lumen with some gasts. The cells of the adrenal glands were swollen and the cytoplasm in some areas had lost its normal granular structure (193).

10.4 Mutagenicity and genotoxicity

No studies on mutagenicity and carcinogenicity of ammonia performed according to current standards are available. Mutagenicity tests of ammonia have been performed in *Escherichia coli*, chick fibroblast cells, and *Drosophila melanogaster*. Positive results were noted in a reverse mutation test in *Escherichia coli*, but only in treatments using toxic levels of NH_4^+ (98% lethality) (43). Slight

mutagenic activity was seen also in *Drosophila* following exposure to ammonia gas, but survival after treatment was less than 2% (114).

Reduced cell division was noted in mouse fibroblasts cultured in media to which ammonia and ammonia chloride were added (188). The effects were pH independent. Decreased rate of DNA synthesis *in vivo* was observed in mouse mucosal cells in the ileum and colon when serum NH_4^+ levels were significantly elevated over normal levels. These elevated levels were induced by intraperitoneal injection of urease or infusion of ammonium chloride (200).

10.5 Effects of long-term exposure and carcinogenicity

Oral exposure to 193 mg ammonium/kg body weight/day as ammonium hydroxide in drinking water for two years did not produce carcinogenic effects in Swiss and C3H mice, and had no effect on spontaneous development of breast adenocarcinoma in C3H females, a characteristic of this strain (177).

No evidence of carcinogenic effects was found in CFLP mice treated intragastrically with ammonia dissolved in water alone at a dose of 42 mg ammonium/kg/day for 4 weeks or with diethyl pyrocarbonate (DEPC) alone, but 9/16 mice treated with a combination of ammonium and DEPC developed lung tumours. The ammonia and DEPC may have reacted *in vivo* to form the carcinogen, urethane, which produced lung tumours in 9/9 of the mice (182). No lung tumours were observed in the offspring of mice exposed similarly to ammonium and DEPC during pregnancy or during lactation (183).

In a concomitant animal study, adult albino male mice (10 exposed, 5 controls) were exposed to vapours of a 12% ammonia solution 15 minutes/day, 6 days/week for 4, 5, 6, 7, or 8 weeks (60). All animals had histological changes in the respiratory tract. In animals sacrificed after four and five weeks, the respiratory epithelium revealed crowding of the cells forming crypts and irregular arrangements. At week six, epithelial hyperplasia was noticed and in four animals of ten exposed, patches of squamous metaplasia were seen. At week seven, three exposed animals showed dysplasia in the nasal epithelium, while a carcinoma *in situ* was detected in one nostril of one animal with loss of polarity of the epithelium, hyperchromatism, and mitotic figures with an intact basement membrane. At week eight, one mouse had an invasive adenocarcinoma of the nasal mucosa. The levels and cell locations of succinic dehydrogenase, acid phosphatase, alkaline phosphatase, and non-specific esterase activities were altered, indicating altered cell metabolism and energy production, cell injury, proliferation and possibly chronic inflammation and neoplastic transformation (60).

Two studies indicate that the ammonium ion may act as a promoter of gastric cancer in rats pretreated with the initiator *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (178, 179). Male Sprague-Dawley rats administered 83 mg/l MNNG in the drinking water for 24 weeks before receiving 0.01% ammonium in the drinking water for 24 weeks had a statistically significantly greater incidence of

gastric cancer (70% of rats) and number of tumours per tumour-bearing rat (2.1) than rats receiving only MNNG and tap water (31% and 1.3 tumours/rat) (178). Additionally, the size, depth, and metastasis of the MNNG-initiated tumours were enhanced by ammonium (179).

These studies suggest that ammonium in the presence of certain other chemicals (i.e. DEPC and MNNG) may contribute to the development of cancer (179, 182).

10.6 Reproductive and developmental studies

No data have been found regarding reproductive and developmental effects in animals after inhalation exposure to ammonia.

In a study by Miñana *et al*, Wistar rats were exposed to ammonia from day 1 of gestation and through the prenatal and lactation periods via a diet containing ammonium acetate (20% by mass) (133). After weaning (at postnatal day 21), the pups were fed a normal diet with no ammonia added. The body weight of offspring exposed to ammonia was significantly lower than that of controls, a difference that was still evident one month after cessation of the exposure. Primary cell cultures of cerebellar neurons from 8-day old offspring exhibited impairment of the NMDA receptor function, as shown by decreased binding of [3H]MK-801, increased resistance to glutamate and NMDA toxicity, and a lack of increase in aspartate aminotransferase activity when small amounts of NMDA were added to culture media (133). A study of similar design by Azorin *et al*, but with adult male rats, observed significantly lower body weight of the male rats maintained on the ammonium diet compared to the controls. Pair feeding showed that this was due to a combination of lower food intake and lower caloric content of the ammonium-enriched feed (13). Maternal body weight was not monitored in the Miñana study, but the results from Azorin and co-workers make it very probable, that maternal body weight was reduced during gestation.

Aguilar *et al* investigated the effect of perinatal hyperammonaemia on active and passive avoidance behaviour and conditional discrimination learning in male Wistar rats (2). Pre- and neonatal exposure to ammonia was carried out as described above for Miñana *et al* (133). However, the exposure to ammonia was continued also after weaning, until and during behavioural testing. Animals exposed to ammonia already during prenatal life exhibited a decreased number of active avoidances on one of five days of testing, and a decreased step-through latency during passive avoidance. These effects were not observed in animals only exposed to ammonia during postnatal life, indicating a prenatal component of ammonia related effects. However, it should be noted that exposure to ammonia during postnatal life was initiated only two weeks before behavioural testing. In comparison, exposure of prenatally exposed animals was continued throughout lactation and some additional weeks until behavioural testing (2).

11. Observations in man

11.1 Irritation and sensitisation

Ammonia irritates the upper airways, eyes, and skin in humans. According to two controlled human exposure studies clear signs of mild respiratory and eye irritation appear somewhere between 25 and 50 ppm (80, 173) (see chapter 11.2 for more details).

Four out of six human subjects described moderate irritation of the nose and eyes when exposed to 50 ppm, but not 30 ppm ammonia gas for 10 minutes (117). When human volunteers were exposed to 100 ppm ammonia in each nostril of the nasal airways for 30 seconds, a significant increase in airway resistance during the exposure period was registered, and 11 out of 23 complained of nasal irritation (128). In another study, human volunteers were exposed to various concentrations of ammonia vapours for 5 minutes. At the concentration of 134 ppm, severe nose irritation occurred in most subjects. At 72 ppm, several subjects reported the same symptoms; at 50 ppm, two subjects reported nasal dryness; and at 32 ppm, one subject reported nasal dryness (97, industrial report).

Ammonia has a greater tendency than other alkalis to penetrate and damage the iris, and to cause cataract in severe burns. Iritis may be accompanied by hypopyon (a layering of white blood cells in the anterior chamber) or haemorrhages, extensive loss of pigment, and severe glaucoma (69, 76, 78). Two cases of ocular injuries, with a rise in intraocular pressure and cataract formation after ammonia (at unknown concentration) had been squirted into the victims' eyes during robberies, were reported (78). In both cases, the more severely affected eyes showed marked injection and oedema of the conjunctivae, diffuse corneal damage, semi-dilated, oval, and fixed pupils. A marked persistent increase in the intra-ocular pressure was seen. An open angle glaucoma and cataract formation were seen in both cases. One drop of a 9% ammonium hydroxide accidentally applied in a patient's eye resulted in an almost complete loss of the corneal epithelium despite irrigation with water initiated within 10 seconds. The eye recovered completely in 3-4 days (70).

Case reports on skin damage following accidental exposure to ammonia are numerous but quantitative data are lacking. Most reports attribute described injuries to the strong alkali effects of ammonia causing chemical burns (7, 196). Direct contact with liquid anhydrous ammonia may cause frostbite in addition to the burns. Anhydrous ammonia in concentrations of 10 000 ppm is sufficient to evoke skin damage (7, 18). However, such high exposure levels are almost certainly fatal if respiratory equipment is not used.

No data on human sensitisation from exposure to ammonia have been located in the current literature.

11.2 Effects of single and short-term exposure

There are several reports in the literature of acute human accidental inhalation exposures to ammonia (26, 34, 63, 131, 194, 199). A review of the early literature on ammonia toxicity cites acute exposure to 5 000-10 000 ppm as being rapidly fatal in humans (134) and exposure to 2 500-4 500 ppm as being fatal in about 30 minutes (76, 132). Elevated levels of metabolites of hydroxylysine have been observed in urine from ammonia accident victims. This indicates collagen degradation secondary to inhalation injury (7).

Immediate deaths resulting from acute exposure to ammonia seems to be caused by airway obstruction while infections and other secondary complications are lethal factors among those who survive for several days or weeks. Chemical burns and oedema of exposed tissues, including the respiratory tract, eyes, and exposed skin are often observed after exposure to lethal levels (12). However, reliable measurements of concentrations and durations of exposure are generally lacking (131).

In a liquefied ammonia production plant of the petrochemical industry, a 47-year-old technician was exposed to ammonia for over 45 minutes after burst of a pipe carrying liquefied ammonia. The patient manifested cutaneous, respiratory, and ocular damage in addition to a severe cold thermal injury (frostbite), had flaccid quadriparesis, and episodes of bradycardia (63). The accident had a fatal outcome. The authors estimated the ammonia concentration to be between 2 000 and 2 500 ppm.

There is one case report on a 48-year old male who inhaled ammonia fumes as a result of a train spill of urea. The patient developed symptomatology of burning of the eyes and mouth, nausea, throat and mouth irritation. This complicated into a state that was diagnosed as ammonia-induced sinusitis (20).

A lifelong non-smoker, and a victim of a massive accidental exposure to anhydrous ammonia gas was followed for 10 years. In the acute phase, the patient presented with severe tracheobronchitis and respiratory failure, caused by very severe burns of the respiratory mucosa. After some improvement he was left with severe and fixed airways obstruction. Isotope studies of mucociliary clearance, computed tomography, and bronchography showed mild bronchiectasis. Thus, acute exposure to high concentrations of ammonia leads to acute inhalation injury but also to long-term impairment of respiratory function (42, 106).

Two painters, (one 41-year old and the other 45-year old with 20 and 25 years work experience, respectively), spray painted in a very poorly ventilated apartment. The paint used was a one-stage vinyl latex primer containing 25% ammonia and 16.6% aluminium chlorohydrate, and a number of other additives. There were no isocyanates or anhydrides. After spray painting for 12 hours, the two painters simultaneously noted the onset of generalised weakness, nausea, cough, shortness of breath, paint taste in their mouths, chest tightness, and wheezing. They were hospitalised for about two weeks with provisional diagnoses of “acute chemical bronchitis”. After discharge from hospital, each subject continued to note persistent wheezing, cough, exertional dyspnoea, and each reported aggravation of symptoms after exposure to non-specific stimuli. At

the University of Cincinnati the illness was designated as reactive airways dysfunction syndrome (RADS), an asthma-like syndrome with persistent bronchial hyperreactivity but no sensitisation (24).

Six volunteers were exposed to 25, 50, and 100 ppm ammonia by inhalation for 2-6 hours/day, 5 days/week for 6 weeks in an ammonium bicarbonate plant (two subjects per exposure level). Suitable locations within the plant were selected to achieve the desired ammonia concentrations. For the 100 ppm exposure level, it was necessary to use a temporary exposure chamber and provide a source of ammonia. Pulse rate, respiration rate, pulmonary functions (forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1)), blood pressure, neurological responses, and interferences in task-performance ability were periodically examined. A statistical analysis of the results demonstrated that the only significant change among the vital functions measured was an increase in FEV_1 with increasing ammonia concentration. Mild eye, nose, and throat irritation was noted after examination by a physician, but an acclimatisation was suggested by the authors as no discomfort was experienced by the subjects after the first week. A clear dose-effect relationship was lacking. During occasional excursions in the range of 150-200 ppm, all subjects experienced some watering of the eyes and a sensation of dryness in the nose and throat (57). The results are difficult to evaluate as the experimental design is unclear, the ammonia levels were not well controlled and only two subjects per exposure level were used.

Sixteen volunteers (eight students with knowledge of the effects of ammonia and eight students not familiar with ammonia health effects) were exposed four at a time to 50, 80, 110 or 140 ppm ammonia for 2 hours. The respiratory variables of vital capacity (VC), forced expiratory volume (FEV), and forced inspiratory volume (FIV), measured before and after exposure, did not decrease by more than 10%. During exposure, each participant recorded subjective effect levels for smell, taste, irritation of eyes, nose, throat or breast, urge to cough, headache and general discomfort every 15 minutes ranking them on a scale ranging from 0 to 5. Subjective responses were ranked higher by those not familiar with the effects. A concentration of 110 ppm was tolerated for 2 hours, but at 140 ppm, all the subjects not familiar with the effects left the chamber because the exposure was intolerable. The subjects experienced only mild irritation to eyes, nose, and throat when exposed to 50 ppm (185).

Six healthy volunteers and eight subjects with mild asthma were exposed to 16-25 ppm ammonia for 30 minutes. Neither healthy subjects nor asthmatics showed any significant changes for pulmonary function (FEV_1 and carbon monoxide diffusing capacity) or bronchial hyperreactivity (metacholine challenge) (163).

Twelve healthy subjects (7 women, 5 men) were randomly exposed to sham or ammonia (0, 5 and 25 ppm) for 3 hours in an exposure-chamber. The exposure to ammonia did not significantly influence lung function, bronchial responsiveness to metacholine, leucocyte counts and complement factors C3 and C3b in blood, total cell count and interleukins 6 and 8 in nasal lavage, and exhaled nitric oxide

levels. The authors reported that these ammonia exposures did not cause detectable upper-airway inflammation or increased bronchial responsiveness. However, all symptom ratings (discomfort in the eyes, nose, throat and airways, breathing difficulty, solvent smell, headache, fatigue, nausea, dizziness, and feeling of intoxication) increased significantly during exposure to 25 ppm ammonia compared to sham exposure. The symptoms remained constant during the exposure, with no signs of adaptation. At 5 ppm, discomfort in the eyes, solvent smell, headache, dizziness, and feeling of intoxication were significantly increased. The average ratings of eye discomfort increased from "Not at all" during sham exposure, to "Hardly at all" (approx. 6 mm on the 100-mm visual analogue scale) at 5 ppm, and "Somewhat" (approx. 20 mm) at 25 ppm ammonia (173). NEG therefore considers 5 ppm as a pragmatic NOAEL and 25 ppm as a LOAEL for irritation.

In another study, 43 healthy males (whereof ten "habituated", i.e. previously exposed to ammonia at work) were exposed 4 hours daily to increasing levels of ammonia on five consecutive days; day 1: 0 ppm, day 2: 10 ppm, day 3: 20 ppm, day 4: 20 ppm and two 30-minutes peaks of 40 ppm, and day 5: 50 ppm. There was no significant trend over time for lacrimation, interleukins 6 and 8 in nasal lavage, nasal resistance, lung function, and bronchial responsiveness to acetylcholine. No effects on cognitive functions such as power of concentration, attention, or reaction time were seen. The perceived intensity of symptoms and annoyance (sum of symptom scores using the Swedish Performance Evaluation System) increased significantly with increasing ammonia levels. The 33 non-habituated tended to rate symptoms higher than the habituated subjects, although this difference was not statistically significant. The rating of olfactory symptoms was significantly increased already at the lowest level of ammonia (10 ppm) among the non-habituated. Irritative and respiratory symptoms were significantly increased in the non-habituated subjects but the exposure levels at which significance was attained cannot be deduced from the report. At 50 ppm, eye irritation (conjunctival hyperaemia) was seen in 3 of 33 non-habituated (9%). In habituated subjects the irritative symptoms increased significantly only at 50 ppm whereas the respiratory symptoms were not significantly increased at any level (80).

11.3 Effects of long-term exposure

Chronic occupational exposure (about 15 years) to low levels of ammonia (9.2 ± 1.4 ppm) (mean ± standard error of mean) had no effect on respiratory or cutaneous symptoms, pulmonary function, or odour sensitivity in 58 male workers at a soda ash factory compared to 31 non-exposed controls (0.3 ± 0.1 ppm) from the same factory (81).

Several studies have been conducted on farmers occupationally exposed to ammonia in enclosed livestock buildings. In addition to ammonia total and respirable dust, carbon dioxide, endotoxins, and microbes were measured (49, 50, 74, 75, 157). Of these pollutants ammonia (2.3-20.7 ppm) and dust (0.04-5.64

mg/m^3) were most frequently associated with respiratory effects, many of which were temporary and disappeared with cessation of exposure. Reynolds *et al* used regression equations for prediction of cross-shift change in pulmonary function based on baseline pulmonary function and environmental exposure to dust, endotoxin, and ammonia (157). The authors calculated the levels of dust (2.5 mg/m^3) and ammonia (7.5 ppm) associated with a significant decrease in FEV_1 over the work-shift among swine production workers. The previous dose-response studies with swine workers resulted in exposure limit recommendations of 7 ppm (47) and 7.5 ppm ammonia (50), based on a 3% decline in FEV_1. The authors emphasised that it was difficult to recommend any maximum concentrations for exposure to ammonia, as correlations of ammonia to pulmonary function were not as consistent as were the correlations of pulmonary functions to total dust (50). Heederik *et al* examined 27 pig farmers and found a relationship between ammonia exposure (5.6 mg/m^3 ± 1.9) (geometric mean ± geometric standard deviation) and decrease (5-10%) in lung function, although the change was generally only of borderline significance (75). Donham *et al* studied a total of 257 poultry workers for respiratory symptoms, pulmonary function, and exposure to dust, endotoxin, and ammonia (49). Significant dose-response relationships studied by correlation and multiple regressions were observed between exposures and pulmonary function decrements over a work shift. Exposure concentrations associated with significant pulmonary function decrements were as follows: total dust: 2.4 mg/m^3, endotoxin: 614 endotoxin unit/m^3 (61.4 ng/m^3), and ammonia 8.4 mg/m^3 (12 ppm). In a later study on the same study population it was calculated that synergy between ammonia levels and airborn dust explained up to 43 and 63% of the decline (respectively for FEV_1 and forced expiratory flow rate between 25 to 75% of FVC) in pulmonary function over the work shift (48). Vogelzang *et al* found a relationship between occupational exposure and bronchial responsiveness in pig farmers and at re-examination three years later the same authors demonstrated increased bronchial responsiveness, which was associated with the exposure to ammonia and significantly associated with the exposure to inhalable dust (189, 190). Most studies reported an association between exposure to pollutants, including ammonia, in livestock confinement buildings and an increase in respiratory symptoms (such as bronchial reactivity/ hyper-responsiveness, inflammation, cough, wheezing or shortness of breath) and a decrease in pulmonary function. The data indicate that ammonia may contribute to transient respiratory distress in exposed farmers but it cannot be concluded to what extent, as the workers were also exposed to other respiratory toxicants, namely dust and endotoxins (49, 50, 129).

A cross-sectional study investigated the prevalence of respiratory symptoms (cough, phlegm, wheezing and dyspnoea) and diseases among 161 employees chronically exposed to ammonia in two urea fertiliser producing factories and 355 unexposed controls (14). The 84 exposed subjects in the factory (A) with ammonia levels ranging between 2 and 130 mg/m^3 (3-182 ppm) had significantly higher relative risks for all respiratory symptoms. The corresponding relative risks

for the 77 subjects in the other factory (B) with considerably lower ammonia levels (0.02-7 mg/m^3 or 0.03-10 ppm) were non-significant. The geometric means for ammonia were below 18 mg/m^3 (25 ppm) except in the urea store and the packing area of factory A with means of 115 and 18.6 mg/m^3 (164 and 26.6 ppm), respectively. Employees in the urea store were not allowed to enter the section unless in full protective clothing. The prevalence rate of haemoptysis was also significantly increased in factory A. All haemoptysis cases were reported to work in areas with high ammonia exposures. Stratification by exposure level in the total material showed significantly increased relative risks for cough (3.48, 95% confidence interval (CI) 1.84-6.57), phlegm (3.75, 95% CI 1.97-7.11), wheezing (5.01, 95% CI 2.38-10.57), dyspnoea (4.57, 95% CI 2.37-8.81) and bronchial asthma (4.32, 95% CI 2.08-8.98) among subjects exposed to average ammonia levels above 18 mg/m^3 (25 ppm). Wheezing was also higher among those exposed at or below 18 mg/m^3 (2.26, 95% CI 1.32-3.88). Similarly, stratification by cumulative ammonia levels showed significantly increased relative risks for all four respiratory symptoms and asthma but also for chronic bronchitis in subjects exposed above 50 mg/m^3-years (70 ppm-years). Wheezing was significantly higher in subjects exposed to cumulative ammonia levels at or below 50 mg/m^3-years (70 ppm-years). Logistic regression analysis revealed that the ammonia concentration was significantly related to cough, phlegm, wheezing with and without shortness of breath, and asthma. Furthermore, the ammonia concentration was the only significant determinant for wheezing with shortness of breath and asthma. The majority of the asthmatics worked in areas with high ammonia concentrations (14). In a later study by the same authors, the effect of ammonia exposure on pulmonary function (FEV_1 and FVC) among 73 workers and 348 unexposed control subjects (most likely part of the study population described above) was investigated (3). FEV_1% predicted and FEV_1/FVC% were lower and FVC% predicted was significantly lower in controls than the workers. However, the workers with high cumulative ammonia exposure (above 50 mg/m^3-years) had significantly lower FVC% predicted and FEV_1% predicted than the workers with lower cumulative exposure. Furthermore, the symptomatic workers (having cough, phlegm, wheezing and/or dyspnoea) had significantly lower FEV_1% predicted and FEV_1/FVC% than the workers without symptoms. After stratification by smoking habit, the FEV_1% predicted remained significantly lower among the symptomatic non-smokers (3).

A 39-year old man with no past record of asthma developed symptoms of cough, breathlessness, and wheezing after five months work with ammonia-containing silver polish in a small poorly ventilated room (107). He could sense a strong ammonia smell during polishing and the ammonia levels in breathing zone were measured to be approximately 8-15 ppm. In addition to ammonia the silver polish also contained isopropyl alcohol, clay, fatty acid, and water. The subject had no symptoms during brass polishing with ammonia levels below 1 ppm. In a controlled experiment, the subject developed rhinitis, tearing, and coughing about 15 minutes after starting polishing of silverware. Rhonchi were detected in both

lungs and the peak expiratory flow rate (PEFR) fell by 42%. Nebulised ventolin was given and PEFR was normalised but fell again 6 hours later. In another experiment, the same patient was exposed to 12 ppm ammonia. Within 2 minutes he had an asthmatic attack and rhonchi were detected in both lungs. Non-specific bronchial hyperreactivity as assessed by histamine provocation was observed (107).

One case report describes a 68-year old male who was exposed to ammonia and developed interstitial pulmonary fibrosis (21). The patient had been employed for 18 years during which time he made photocopies and operated developing equipment and cameras. He had been exposed frequently to anhydrous ammonia leaks and described the odour of ammonia during these leaks. Throughout the patient's years of employment there were documented complaints about smell of ammonia and symptoms during episodes of ammonia leaks at 30-400 ppm. The ammonia concentrations in breathing zone samples taken during cylinder change and where ammonia was sprayed ranged from 0-2 ppm and 15-20 ppm, respectively (21).

The acute and chronic lung conditions that have been reported in association with ammonia exposure are listed in Table 8. According to de la Hoz *et al* there are few pathophysiologically well-characterised chronic pulmonary diseases due to massive exposures to ammonia, and clinical follow-up for at least one year is available for only a minority of the cases reported in the literature (42). This is in part due to the acute mortality: death occurred instantaneously or in hospital within 60 days of the exposure in 20 of the 94 reported cases examined by de la Hoz *et al* (42). Death usually resulted from severe laryngeal oedema and obstruction, non-cardiogenic pulmonary oedema, or extensive pneumonic complications. Inhalation injury has been infrequently associated with chronic interstitial lung disease, with restrictive ventilatory pattern but case reports and indirect epidemiological studies have described interstitial lung disease from ammonia exposure (21).

Table 8. Summary of pulmonary conditions associated with ammonia inhalation injury in case reports. Adapted from de la Hoz *et al* (42).

Conditions	Reference
Acute	
Pulmonary oedema	(34, 44, 168)
Laryngitis/tracheobronchitis (with or without obstruction)	(34, 106)
Bronchiolitis	(168, 191)
Bronchopneumonia	(44, 58, 168)
Reactive airways dysfunction syndrome (RADS)	(24)
Chronic	
Bronchiectasis	(34, 94, 106)
Bronchospasm/asthma	(17, 58)
Bronchiolitis	(94, 168, 191)
Chronic obstructive pulmonary disease (COPD)	(22, 111)
Interstitial pulmonary fibrosis	(21)

11.4 Genotoxic effects

Only one study was found in the current literature on the genotoxic effect of ammonia on humans (198). Comparison of blood samples from 22 workers exposed to ammonia in a fertiliser factory and 42 control workers not exposed to ammonia showed increased frequency of chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs), increased mitotic index, and increased frequency of CAs and SCEs with increasing length of exposure. The length of workers' exposure was 0-5 years (9 persons), 6-10 years (6 persons), and over 11 years (7 persons). In addition to ammonia, nitrogen fertiliser production involves exposure to various nitrate compounds, which may have affected the results.

11.5 Carcinogenic effects

No data on carcinogenic effects of ammonia on humans were found in the literature.

11.6 Reproductive and developmental effects

No data on reproductive and developmental effects of ammonia on humans were found in the literature.

12. Dose-effect and dose-response relationships

12.1 Animal studies

Exposure to an ammonia concentration of 57 ppm for 60-114 days did not lead to any sign of toxicity or lung abnormalities in any of the animal species tested (5, 35). Histological changes in the lungs and respiratory tract appeared in rats after 4-75 days exposure to 143-200 ppm ammonia (5, 23, 61). The RD_{50} value in mice used as a measure of respiratory irritation in humans was calculated to be 257-303 ppm (15, 201). Dose-effect relationships after exposure to ammonia via inhalation for experimental animals are listed in Table 9.

12.2 Human studies

According to two controlled human exposure studies (3-4 hours) clear signs of mild respiratory and eye irritation appear somewhere between 25 and 50 ppm (80, 173). No upper-airway inflammation or increased bronchial responsiveness was seen at these levels. Chamber exposures to a concentration of 110 ppm was tolerated for 2 hours, but at 140 ppm, all subjects not familiar with the effects left the chamber because the exposure was intolerable (185).

Chronic occupational exposure to low levels of ammonia (9.2 ± 1.4 ppm)

Table 9. Dose-effect relationships for experimental animals after exposure to ammonia via inhalation.

Dose		Species	No. of animals	Duration	Effect	Reference
mg/m^3	ppm					
18	25	Rat (Fisher)	12	7 days + 35-42 days, continuous exposure	Rats inoculated with *Mycoplasma pulmonis* intranasally. Increased severity and prevalence of lesions characteristic of mycoplasmosis.	(23)
40	57	White rat	–	2 months	Threshold level for toxic effects.	(5)
40	57	Rat[a] Guinea pig Rabbit Dog Monkey	15 15 3 2 3	114 days, continuous exposure	No toxic signs and gross or microscopic lung abnormalities in any species.	(35)
63	90	Guinea pig	24	3 weeks, continuous exposure	Guinea pigs vaccinated with *Myco-bacterium bovis* and challenged with derivative of tuberculin. Decrease in the cell-mediated immune response.	(175)
85	121	White rat	–	2 hours	Threshold level for acute effects as depression, then hyperactivity and convulsions.	(5)
100	143	White rat	–	2 months	Histological changes in lungs as small areas interstitial pneumonia.	(5)
100	143	White rat	–	60 min	No effects observed.	(154)
105	150	Rat (Sherman)	10	75 days, continuous exposure	Histological changes (increased thickness and hyperplasia) in respiratory and olfactory epithelium.	(23)
119	170	Guinea pig	12	6 h/day, 5 days/week up to 18 weeks	Mild changes in spleens, kidneys, liver, and adrenal glands.	(193)
127	181	Rat[a]	48	90 days, continuous exposure	No changes in gross or microscopic pathology, haematology, liver histochemistry.	(35)

Table 9. Cont.

Dose mg/m^3	Dose ppm	Species	No. of animals	Duration	Effect	Reference
140	200	Rat	–	4-12 days, continuous exposure	Histopathological changes in the tracheal epithelium including loss of cilia and hyperplasia.	(61)
180	257	Mouse (Swiss OF_1)	–	15 min	RD_{50}	(201)
212	303	Mouse (Swiss-Webster)	12	30 min	RD_{50}	(15)
262	374	Rat[a]	49	90 days, continuous exposure	Mild nasal discharge in 25% of the animals.	(35)
300	431	White rat	–	60 min	Decrease in static muscle tension, leukocytosis, prolongation of latent reflex time, increase in total protein and blood sugar, increased oxygen consumption, and rise in the level of residual nitrogen.	(154)
455	650	Rat[a]	51	65 days, continuous exposure	1/51 survivor. All 51 showed mild dyspnoea and nose irritation.	(35)
470	671	Rat[a]	15	90 days, continuous exposure	2/15 survivors.	(35)
		Guinea pig	15		11/15 survivors.	
		Rabbit	3		3/3 survivors, erythema, discharges, and opacity of cornea in 3/3, moderate lung congestion in 2/3.	
		Dog	2		2/2 survivors, heavy lacrimation, and nasal discharge in 2/2, haemorrhagic lung lesion in 1/2.	
		Monkey	3		3/3 survivors.	

[a] Both Sprague-Dawley and Long-Evans rats were exposed but information about which species were used for a certain exposure was not given.
Abbreviation: RD_{50}: concentration, which produce a 50% decrease in respiratory rate.

Table 10. Dose-effect relationships in man after exposure to ammonia via inhalation.

Concentration		Duration	No. of exposed	Effect	Reference
mg/m^3	ppm				
3.5	5	180 min	12	No upper-airway inflammation or increased bronchial responsiveness. Increased symptom ratings for discomfort in the eyes, solvent smell, headache, dizziness, and feeling of intoxication. Ratings correspond to "Hardly at all".	(173)
6.4	9.2[a]	Chronic exposure	58	No effects on respiratory or cutaneous symptoms, pulmonary function, or odour sensitivity.	(81)
0.02-7.0	0.03-9.8	Chronic exposure	77	No effects on respiratory symptoms.	(14)
7-14	10-20	240 min	43	Increased symptom ratings in 33 non-habituated volunteers for: sum of symptom scores, and olfactory symptoms.	(80)
8.4	12	2 min	1	Asthma, rhonchi in both lungs.	(107)
11-18	16-25	30 min	6 + 8	Neither healthy subjects nor asthmatics showed significant change in pulmonary function or bronchial hyperreactivity.	(163)
18	25	180 min	12	No upper-airway inflammation or increased bronchial responsiveness. Increased rating for all symptoms: discomfort in the eyes, nose, throat and airways, breathing difficulty, solvent smell, headache, fatigue, nausea, dizziness and feeling of intoxication. Irritation ratings correspond to "Somewhat".	(173)
≤18	≤25[b]	Chronic exposure	138	Increased relative risk (95% CI) for wheezing 2.26 (1.32-3.88).	(14)
>18	>25[b,c]	Chronic exposure	17	Increased relative risk (95% CI) for cough 3.48 (1.84-6.57), wheezing 5.01 (2.38-10.57), phlegm 3.75 (1.97-7.11), dyspnoea 4.57 (2.37-8.81), bronchial asthma 4.32 (2.08-8.98).	(14)
14 and 28	20 and 40	240 min and 2 x 30 min	43	Increased symptom ratings in 33 non-habituated volunteers for sum of symptom scores, olfactory symptoms, irritative symptoms.	(80)
21	30	10 min	5	No irritation in 3/5 and "just perceptible" irritation of eyes and nose in 2/5.	(117)

Table 10. Cont.

Concentration mg/m^3	Concentration ppm	Duration	No. of exposed	Effect	Reference
35	50	10 min	6	"Moderate" irritation of eyes and nose in 4/6.	(117)
35	50	240 min	43	Increased symptom ratings for sum of symptom scores, olfactory symptoms, irritative symptoms. Conjunctival hyperaemia in 3 of 33 (9%) non-habituated.	(80)
35-56	50-80	120 min	16	VC, FEV, and FIV did not decrease more than 10%. Mild irritation in eyes and throat.	(185)
70	100	5-30 s	23	Increased nasal airway resistance during the exposure periods. Nasal irritation in 11/23.	(128)
77	110	120 min	16	VC, FEV, and FIV did not decrease more than 10%. Irritation in eyes and throat, cough.	(185)
98	140	≤120 min	16	VC, FEV, and FIV did not decrease more than 10%. Intolerable for 8/16.	(185)
>105	>150		6	All subjects experienced lachrymation accompanied by dryness of the nose and throat during occasional excursions above 150 ppm in semi-controlled exposures to ammonia in an ammonium bicarbonate plant.	(57)
1 190[d]	1 700[d]	Accident		Coughing and laryngospasm along with oedema of the glottic region.	(7)
1 750-3 160[d]	2 500-4 500[d]	Accident		Fatal in approximately 30 min.	(7)
7 000[d]	10 000[d]	Accident		Rapid respiratory arrest. Anhydrous ammonia in concentrations of 10 000 ppm sufficient to evoke skin damage.	(18)

[a] Time-weighted average, [b] Geometric mean, [c] Maximal exposure level 130 mg/m^3 (185 ppm), [d] Retrospective estimates.
Abbreviations: CI: confidence interval, VC: vital capacity, FEV: forced expiratory volume, FIV: forced inspiratory volume.

(mean ± standard error of mean) had no effect on respiratory or cutaneous symptoms, pulmonary function, or odour sensitivity (81). In another study, no signs of respiratory symptoms were seen in workers exposed to ammonia concentrations between 0.03 and 9.8 ppm. However, workers exposed to average ammonia levels at or below 25 ppm had an increased relative risk for wheezing. Additionally, the workers exposed to average levels above 25 ppm had higher relative risks for wheezing, cough, phlegm, dyspnoea, and bronchial asthma (14). One case report describes a man who developed asthma-like symptoms after five months exposure to low levels of ammonia (8-15 ppm) from ammonia containing silver polish. Subsequent experimental exposure to 12 ppm produced an immediate asthmatic reaction (107). Dose-effect relationships after exposure to ammonia via inhalation for humans are shown in Table 10.

13. Previous evaluations by national and international bodies

NEG concluded in 1986 that the significant effect of exposure to ammonia is irritation of the respiratory tract, skin, and eyes. Studies of human inhalation of ammonia at low or moderate concentrations (less than 50 mg/m^3 = 70 ppm) had shown mild eye and upper respiratory tract irritation. Exposure to high concentrations of ammonia (by accidental release) may result in skin burns, eye damage, respiratory injury, and death (110).

IPCS concluded that exposure to ammonia in air at a concentration of 390 ppm (280 mg/m^3) produced throat irritation; 1 680 ppm (1 200 mg/m^3) produced cough; 2 380 ppm (1 700 mg/m^3) was life-threatening, and more than 4 900 ppm (3 500 mg/m^3) was associated with high mortality. Respiratory symptoms were usually reversible, but chronic bronchitis had been reported to develop. Irritation occurred at 50 ppm (35 mg/m^3), which was neither discomforting nor painful. However, the report further concluded that ammonia does not present a direct threat to man except as a result of accidental exposure, particularly in industrial settings (87).

The Health and Safety Executive (HSE), United Kingdom, summarised that the only clearly established effect arising from exposure to low concentrations (less than 200 ppm) of ammonia in humans is irritation of the skin, eyes, and upper respiratory tract. In unaccustomed subjects, clear indications of irritation were reported with exposure to 50-55 ppm for periods between several or 10 minutes and 6 hours. There is some evidence that repeated exposure to ammonia may lead to some degree of acclimatisation. The HSE report concluded that from the very limited data available there is no clear evidence that ammonia possesses genotoxic, carcinogenic, or reprotoxic potential (147).

Ammonia has not been classified for carcinogenic effects by United States Environmental Protection Agency (EPA), United States Department of Health and Human Services/National Toxicology Program (DHHS/NTP) or the International Agency for Research on Cancer (IARC).

In the criteria document for ammonia by the Commission of the European Communities (1993) the current occupational exposure limit of 17 mg/m^3 (25 ppm) was considered a borderline value. Therefore, an exposure limit of 14 mg/m^3 (20 ppm) was suggested. If a short-term exposure limit (STEL) value will be considered necessary, a level of 22 mg/m^3 (32 ppm) was suggested (62).

The Agency for Toxic Substances and Disease Registry (ATSDR) has derived a minimal risk level (MRL), of 1.7 ppm for acute-duration inhalation exposure, and a MRL of 0.1 ppm for chronic-duration inhalation exposure to ammonia (12).

The American Conference of Governmental Industrial Hygienists (ACGIH) has recommended an 8-hour occupational exposure limit of 25 ppm and a STEL of 35 ppm for ammonia. The first value is recommended to minimise the potential eye and respiratory tract irritation and the second one to provide a greater margin of safety against the acute sensory effects from exposure to ammonia (1).

14. Evaluation of human health risks

14.1 Assessment of health risks

Ammonia is an endogenous compound formed and used in various metabolic reactions in the human body. The most serious effects of elevated endogenous ammonia are seen in the brain as hepatic encephalopathy after acute or chronic liver failure. At the cellular level, ammonia interacts with various cellular processes involved in neurotransmission and brain energy metabolism.

The direct local action on mucous membranes results in irritation and inflammation of the skin, eyes, nose, throat, and upper respiratory tract. Acute contact with high concentrations of ammonia can produce rapid changes in the pulmonary parenchyma due to the affinity of the anhydrous by-products for mucous membranes leading to death. Clinically, mild insults produce coughing as well as laryngitis and tracheobronchitis. Ammonia inhalation injury has also been associated with RADS, an asthma-like syndrome with persistent bronchial hyperreactivity but no sensitisation. More severe injuries include in fulminant pulmonary oedema and bronchiectasis. The most frequent injury is desquamation of the epithelial layer of the upper tracheobronchial tree.

Ammonia can penetrate and damage the iris, and cause cataract in severe burns. Iritis may be accompanied by hypopyon (a layering of white blood cells in the anterior chamber) or haemorrhages, extensive loss of pigment, and severe glaucoma.

Chronic occupational exposure to low levels of ammonia (9.2 ± 1.4 ppm) (mean ± standard error of mean) had no effect on pulmonary function or odour sensitivity in a group of workers. In another study, chronic exposure to average ammonia levels above 25 ppm was associated with increase in respiratory symptoms and bronchial asthma.

According to two controlled human exposure studies clear signs of mild respiratory and eye irritation appear somewhere between 25 and 50 ppm. In one

of the studies, significant increases in symptom ratings were seen already at 5 ppm, however, the ratings corresponded verbally to “Hardly at all”. NEG considers 5 ppm as a pragmatic NOAEL and 25 ppm as a LOAEL for irritation. No upper-airway inflammation or increased bronchial responsiveness were detected at these levels.

RD_{50} values for ammonia calculated from mouse studies are 260-300 ppm. A NOAEL of 57 ppm and a LOAEL of 143 ppm for toxic effects were reported in a 2-month inhalation exposure of white rats. The NOAEL for irritation in humans is often considered as approximately 3% of the RD_{50}, i.e. 9 ppm ammonia.

14.2 Groups at extra risk

Ammonia is commonly regarded as an irritant. Therefore, persons who are hyper-reactive to other respiratory irritants or who are asthmatic, would also be more sensitive to ammonia. As ammonia is metabolised and excreted by liver and kidney, persons with hepatic or renal impairment might be at risk when exposed to ammonia as well as those with inherited deficiencies of urea cycle enzymes.

14.3 Scientific basis for an occupational exposure limit

Based on short-term (3-4 hours) controlled exposure of volunteers, the critical effect of exposure to ammonia is irritation. According to these studies clear signs of mild respiratory and eye irritation appear somewhere between 25 and 50 ppm. In one of the studies, significant increases in symptom ratings were seen already at 5 ppm, however, the ratings corresponded verbally to “Hardly at all”. NEG considers 5 ppm as a pragmatic NOAEL and 25 ppm as a LOAEL for irritation. No upper-airway inflammation or increased bronchial responsiveness were detected at these levels. Chronic occupational exposure to low levels of ammonia (9.2 ± 1.4 ppm) (mean ± standard error of mean) had no effect on pulmonary function or odour sensitivity. In another study, chronic exposure to average ammonia levels above 25 ppm was associated with an increase in respiratory symptoms and bronchial asthma.

15. Research needs

Long-term animal tests are needed to establish a NOAEL for inhalation exposure. There is a lack of data on health effects and exposure levels from long-term and low exposure situations where ammonia is the principal agent. More studies on health effects are also needed from persons who have been exposed to high levels of ammonia. In addition, more studies on reproductive toxicity and carcinogenicity should be undertaken to provide proper data for risk assessment.

16. Summary

Liesivuori J. *The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals*. 137. Ammonia. Arbete och Hälsa 2005;13:1-52.

Ammonia (NH_3) is a colourless gas with a distinctly pungent odour at normal atmospheric temperatures and pressures. The odour threshold is approximately 5 ppm. Ammonia can be liquefied under pressure. In aqueous solution, NH_3 acts as a base yielding ammonium (NH_4^+) and hydroxide (OH^-) ions. Because of the high water solubility ammonia dissolves in moisture on the mucous membranes, eyes and skin, forming ammonium hydroxide, which may cause alkali burns and liquefaction necrosis of the tissues.

Ammonia occurs naturally in the environment and is endogenously produced in humans and other mammalians. It is also man-made in vast quantities. Occupational exposures may occur in ammonia plants, fertiliser manufacturing, and animal production. The primary route of occupational exposure to ammonia is inhalation, although dermal exposure may also occur during handling of liquid ammonia or aqueous ammonia solutions.

The critical effect of exposure to ammonia is irritation. In controlled human exposure studies (3-4 hours) clear signs of mild respiratory and eye irritation appear somewhere between 25 and 50 ppm. In one of these studies very slight irritation was seen at 5 ppm. NEG considers 5 ppm as a pragmatic NOAEL and 25 ppm as a LOAEL for irritation. Chronic exposure to average ammonia levels above 25 ppm has been associated with an increase in respiratory symptoms (cough, wheezing, phlegm, and dyspnoea) and bronchial asthma. High acute exposure levels may result in reactive airways dysfunction syndrome (RADS), an asthma-like syndrome with persistent bronchial hyperreactivity but no sensitisation. Massive exposure to ammonia can cause eye damage, skin burns, severe inflammation of the respiratory tract (laryngitis, tracheobronchitis, and pulmonary oedema), and death.

With very limited data available, there is no evidence that ammonia possesses genotoxic, carcinogenic or reprotoxic potential.

Keywords: ammonia, asthma, burns, irritation, occupational exposure limit, RADS, review, toxicity

17. Summary in Swedish

Liesivuori J. *The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals*. 137. Ammonia. Arbete och Hälsa 2005;13:1-52.

Ammoniak (NH_3) är en färglös gas med en påtagligt stickande lukt vid normal temperatur och tryck. Lukttröskeln har angivits till ungefär 5 ppm. Ammoniak kondenserar till vätska vid högt tryck. Ammoniak är en svag bas som i vatten bildar ammonium- (NH_4^+) och hydroxidjoner (OH^-). Den höga vattenlösligheten gör att ammoniak löser sig i slemhinnor, ögon och hud och bildar ammonium-hydroxid som i sin tur kan orsaka frätskador och nekros.

Ammoniak förekommer naturligt i vår omgivning och bildas endogent hos människor och andra däggdjur. Ammoniak framställs också industriellt i stora kvantiteter. Förutom vid råvaru- och handelsgödselproduktion förekommer ammoniakexponering framförallt inom jordbruket i samband med djurhållning. Yrkesmässig exponering för ammoniak sker främst genom inhalation. Hudupptag kan ske vid hantering av flytande ammoniak eller vattenlösningar av ammoniak.

Den kritiska effekten vid exponering för ammoniak är irritation. I kontrollerade exponeringsstudier på människa (3-4 timmar) uppträder tydliga tecken på mild irritation i övre luftvägar och i ögon någonstans mellan 25 och 50 ppm. I en av dessa studier noterades en mycket svag irritation vid 5 ppm. NEG bedömer 5 ppm som en pragmatisk icke-effektnivå (NOAEL) och 25 ppm som lägsta observerade effektnivån (LOAEL) för irritation. Kronisk exponering för en genomsnittlig ammoniaknivå över 25 ppm har rapporterats vara förenat med en ökning av luftvägssymptom (hosta, pipande andning, slem och andnöd) och bronkiell astma. Akut exponering för höga halter ammoniak kan ge upphov till ett astma-liknande sjukdomstillstånd, s k RADS (reactive airways dysfunction syndrome) som kännetecknas av kvarstående bronkiell hyperreaktivitet men ingen sensibilisering. Massiv exponering för ammoniak kan orsaka frätskador på hud och ögon, allvarlig inflammation i luftvägarna (laryngit, trakeobronkit och lungödem) och leda till döden.

Det går inte att bedöma om ammoniak har genotoxisk, carcinogen eller reproduktiontoxisk potential med tillgängliga data.

Nyckelord: ammoniak, astma, frätskador, irritation, hygieniskt gränsvärde, RADS, toxicitet, översikt

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19. Data bases used in the search for literature

In the search for literature the following data bases were used:

Arbline
Chemical Abstracts
HSELINE
Medline
NIOSHTIC
Toxline
PubMed (National Library of Medicine)

Last search was performed in April 2005.

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Appendix

Occupational exposure limits for ammonia in air.

Country (Organisation)	ppm	$\mu g/m^3$	Comments	Year	Reference
Denmark	20	14		2005	(1)
Finland	20	14		2005	(2)
	50	36	STEL, 15 min,	2005	(2)
Germany	20	14		2005	(3)
Iceland	25	18		1999	(4)
	50	35	STEL, 5 min	1999	(4)
Netherlands	20	14		2005	(5)
	50	36	STEL, 15 min	2005	(5)
Norway	25	18		2003	(6)
Sweden	25	18		2005	(7)
	50	35	STEL, 5 min	2005	(7)
USA (ACGIH)	25			2005	(8)
	35		STEL, 15 min	2005	(8)
(NIOSH)	25	18		2005	(9)
	35	27	STEL, 15 min	2005	(9)
(OSHA)	50	35		2005	(9)

STEL: short-term exposure limits.

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