Environmental Health Criteria 54

AMMONIA

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World Health Organization

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

ENVIRONMENTAL HEALTH CRITERIA 54

AMMONIA

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The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by the IPCS include the development of know-how for coping with chemical accidents, coordination of laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

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NOTE TO READERS OF THE CRITERIA DOCUMENTS

Every effort is made to present information in the criteria documents as accurately as possible. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors that may have occurred to the Manager of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda, which will appear in subsequent volumes.

* * *

A data profile and information on the various limits set by countries can be obtained from the International Register of Potentially Toxic Chemicals, Palais des Nations, 1211 Geneva 10, Switzerland (Telephone No. 988400 - 985850).

Concentrations in this document are expressed in the terms used in original references.

1 mmol is equivalent to 14 mg ammonia-nitrogen/litre

17 mg NH_3/litre
18 mg NH_4+/litre

1 mg ammonia-nitrogen is equivalent to 1.21 mg NH_3
1.29 mg NH_4+

In air, 1 mg/m^3 is equal to about 1.42 ppm, depending on the temperature and pressure.

ENVIRONMENTAL HEALTH CRITERIA FOR AMMONIA

Following the recommendations of the United Nations Conference on the Human Environment held in Stockholm in 1972, and in response to a number of resolutions of the World Health Assembly and a recommendation of the Governing Council of the United Nations Environment Programme, a programme on the integrated assessment of the health effects of environmental pollution was initiated in 1973. The programme, known as the WHO Environmental Health Criteria Programme, has been implemented with the support of the Environment Fund of the United Nations Environment Programme. In 1980, the Environmental Health Criteria Programme was incorporated into the International Programme on Chemical Safety (IPCS), a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The Programme is responsible for the publication of a series of criteria documents.

A WHO Task Group on Environmental Health Criteria for Ammonia was held in Geneva on 8-13 July, 1985. Dr E.M. Smith opened the meeting on behalf of the Director-General. The Task Group reviewed and revised the draft criteria document and made an evaluation of the health risks of exposure to ammonia.

The original draft of this document was prepared by THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE under the direction of DR J.F. STARA. Additional contributions were made by DR J.R. JACKSON, PROFESSOR D. RANDALL, and DR R.V. THURSTON.

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1. SUMMARY

1.1. Properties and Analytical Methods

Ammonia (NH_3) is a colourless acrid-smelling gas at ambient temperature and pressure. It can be stored and transported as a liquid at a pressure of 10 atm at 25 °C.

Ammonia dissolves readily in water where it forms, and is in

equilibrium with, ammonium ions (NH_4+). The sum of ammonia and ammonium concentrations is termed "total ammonia" and, because of the slightly different relative molecular masses, may be expressed as "total ammonia-nitrogen (NH_3-N)". In most waters, NH_4+ predominates, but increases in pH or temperature or decreases in ionic strength may materially increase levels of non-ionized ammonia.

Ammonia will adsorb on various solids. At concentrations of between 16 and 27% by volume, it can form explosive mixtures with air. Catalytic oxygenation is an important reaction in the manufacture of nitric acid. Ammonia dissolves in dilute acids to form ionized ammonium salts, which are similar in solubility to alkali metal salts, and can be crystallized. Some of these salts are found in nature. Heating solutions or crystals of the salts yields gaseous ammonia. Ammonia forms chloramines in water containing hypochlorous acid.

There are difficulties in sampling media for the determination of ammonia, and in preventing contamination and losses before analysis. A variety of analytical techniques are available; many have interactions. For measurements, the flourescent derivatization technique has advantages.

1.2. Sources in the Environment

Ammonia is present in the environment as a result of natural processes and industrial activity, including certain types of intensive farming. Atmospheric ammonia is volatilized from the earth's surface in quantities of about 10^8 tonnes/year, mostly from natural biological activity. Industrial activity may cause local and regional elevations in emission and atmospheric concentrations. Surface waters receive ammonia from point sources, such as effluent from sewage treatment and industrial plants, in quantities estimated in the USA to be about half a million tonnes annually. Much more significant quantities arise from non-point sources, such as atmospheric deposition, the breakdown of vegetation and animal wastes, applied artificial fertilizers and urban runoff, and these are significant, even in industrial areas.

1.3. Environmental Transport, Distribution, and Transformation

Ammonia in the environment is a part of the nitrogen cycle. It volatilizes into the atmosphere where it may undergo a variety of reactions. Photolytic reactions destroy some of the ammonia and reactions with sulfur dioxide or ozone produce aerosols, most importantly of ammonium sulfate or nitrate, which return to the earth's surface as wet or dry deposition. In surface waters, ammonium may undergo microbiological nitrification, which yields hydrogen and utilizes oxygen so that, in certain systems, acidification and oxygen depletion may result. In one study, one-third of the acidifying effect of precipitation was attributed to ammonium deposition. Ammonia may be assimilated by aquatic plants as a nitrogen source or transferred to sediments or volatilized. In soil, major sources of ammonia are the aerobic degradation of organic matter and the application and atmospheric deposition of synthetic fertilizers. The ammonium cation is adsorbed on positively charged clay particles and is relatively immobile. Most ammonium undergoes nitrification; the nitrate ion is mobile and is removed by leaching, plant root uptake, or denitrification.

1.4. Environmental Levels and Human Exposure

Atmospheric concentrations vary according to underlying land usage. Urban concentrations are typically in the range of 5 - 25 µg/m^3 and rural concentrations, 2 - 6 µg/m^3. Areas with intensive manure production or use may produce concentrations of 100 - 200 µg/m^3. Particulate ammonium concentrations above oceans, remote from land, have been found to be 10 - 115 ng/m^3. In most situations, atmospheric particulate ammonium concentrations are comparable to gaseous ammonia concentrations.

Surface waters contain concentrations of total ammonia that vary both regionally and seasonally. In the USA, most surface waters contain less than 0.18 ng/litre, though those near large metropolitan areas may contain 0.5 ng/litre, as total ammonia. In hydrologically isolated acidified small lakes, concentrations may reach 3 mg NH_4+-N/litre, and values near intensive farms of 12 mg NH_4+-N/litre have been recorded. Ground water usually contains low concentrations of ammonia, because of ammonium adsorption and/or nitrification; this, and the conversion of ammonia to chloramines on chlorination, results in low levels of ammonia in most treated drinking-water.

Ammonia in soil is largely fixed; that in solution is in dynamic equilibrium with nitrate and is not directly available to plants. Ammonia occurs in unprocessed foods, but ammonium salts are added to processed foods. Acceptable Daily Intakes (ADIs), where specified, relate to the anion. Cigarette smoking and certain medicines may contribute to intake, in some cases, but the intake from all sources is small in comparison with endogenous intestinal ammonia production.

Occupational exposure to low levels of ammonia is common, but, in certain occupations, work-place concentrations may exceed 100 mg/m^3. At such levels, the daily ammonia intake is small in relation to endogenous production, but it is significant, since inhaled ammonia enters the systemic circulation.

1.5. Kinetics and Metabolism

1.5.1. Uptake and absorption

At low concentrations, inhaled ammonia dissolves in the mucous fluid lining the upper respiratory tract and little reaches the lower airways. Initial retention is about 80% in both the dog and man, but, in man, it falls to less than 30% in less than 27 min. In rats, increases in blood-ammonia were measured following short-term exposure to ammonia at 220 mg/m^3 but not at 23 mg/m^3. The increases were less marked with longer exposure. Calculated blood-ammonia increases with exposure to air containing 18 mg/m^3 are about 10% of fasting levels.

Ammonia is formed in the human intestinal tract by the biological degradation of nitrogenous matter, including secreted urea, in quantities of about 4 g/day. Nearly all of this is absorbed (mainly passively) and is metabolized in the liver on first passage, so that only small amounts reach the systemic circulation.

1.5.2. Distribution

Ammonia is normally present in all tissues constituting a metabolic pool. Its distribution is pH dependent, since NH_3 diffuses more easily than NH_4+. Oral administration of ammonium chloride to healthy male and female volunteers at 9 mg/kg body

weight produced transient increases in blood-ammonia in about half of the subjects. Patients with cirrhosis showed a greater and more prolonged increase over a higher baseline. This confirms substantial first pass metabolism in the liver.

Administration of ^{15}N-labelled ammonium compounds to experimental animals indicated that the initial distribution of ^{15}N depended on the route of administration and that, after parenteral administration, more was distributed to organs other than the liver.

1.5.3. Metabolic transformation

Ammonia is taken up by glutamic acid in many tissues, and this will take part in a variety of transamination and other reactions, the nitrogen being incorporated in non-essential amino acids. In the liver, ammonia is used in the synthesis of protein by the Krebs-Henseleit cycle.

1.5.4. Excretion and turnover

The principal means of ammonia excretion varies between phyla. Mammals excrete urea and secrete ammonium in the kidney tubules as a means of hydrogen ion excretion. Faecal and respiratory excretion are insignificant. Exhaled air may contain volatilized ammonia from the microfloral degradation of salivary urea. In man, on a 70 g protein/day diet, 70% of administered ammonium ^{15}N is lost in a week; on a 20 g protein/day diet, 35% is lost.

1.5.5. Plant metabolism of ammonia

Ammonia is toxic in plants and cannot be excreted. It is detoxified by combination with carbon skeletons, and so excess ammonia may strain carbohydrate metabolism. Some plants have special means of handling ammonia, enabling them to tolerate it or use it preferentially.

1.6. Effects on Aquatic Organisms

Concentrations of ammonia that are toxic for aquatic animals are generally expressed as non-ionized ammonia (NH_3), because, in the environment, NH_3 and not the ammonium ion (NH_4+) has been demonstrated to be the principal toxic form of ammonia.

Concentrations of ammonia, acutely toxic for fish, can cause loss of equilibrium, hyperexcitability, increased breathing, cardiac output, and oxygen uptake, and, in extreme cases, convulsions, coma, and death. At lower concentrations, ammonia produces many effects in fish including a reduction in egg hatching success, a reduction in growth rate and morphological development, and pathological changes in the tissue of the gills, liver, and kidney.

Several factors have been shown to modify acute ammonia toxicity in fresh water. Some factors alter the concentration of NH_3 in the water by affecting the aqueous ammonia equilibrium, while other factors affect the toxicity of NH_3 itself, either ameliorating or exacerbating its effects. Factors that have been shown to affect ammonia toxicity include dissolved oxygen concentration, temperature, pH, previous acclimatization to ammonia, fluctuating or intermittent exposures, carbon dioxide concentration, salinity, and the presence of other toxic substances. The best studied of these is pH; the acute toxicity of NH_3 has been shown to increase as pH decreases. Data on

temperature effects on acute NH_3 toxicity are limited and variable, but there are indications that NH_3 toxicity is greater at low (< 10 °C) temperatures.

Data concerning concentrations of NH_3 that are toxic for fresh-water phytoplankton and vascular plants, although limited, indicate that fresh-water plant species are appreciably more tolerant to NH_3 than invertebrates or fish.

(a) *Fresh-water organisms*

Mean 48- and 96-h LC_{50} values reported for fresh-water invertebrates and fish ranged from 1.10 to 22.8 mg NH_3/litre for invertebrate species, and from 0.56 to 2.48 mg/litre for fish species. Mean 96-h LC_{50} values ranged from 0.56 to 2.37 mg NH_3/litre for salmonid fish and from 0.76 to 2.48 mg/litre for non-salmonids. In terms of LC_{50}, Percidae and Salmonidae are considered to be the most sensitive families and walleye and rainbow trout are the most sensitive species within these families.

For fresh-water organisms, the families most sensitive in terms of chronic toxicity are Salmonidae and Catostomidae, pink salmon and white sucker being the most sensitive species within these families. Limited chronic toxicity data for invertebrates, mostly cladocerans and one insect species, indicate that they are generally more tolerant than fish, although the fingernail clam appears to be as sensitive as salmonids.

(b) *Salt-water organisms*

Available acute and chronic ammonia toxicity data for salt-water organisms are very limited. Mean LC_{50} values for marine invertebrate species range from 0.94 to 18.3 mg NH_3/litre and, for marine fish species, from 0.32 to 1.31 mg/litre. The prawn, *Macrobrachium rosenbergii,* appears to be the most sensitive invertebrate species tested, and the red drum, the most sensitive fish species.

1.7. Effects on Experimental Animals and *In Vitro* Test Systems

1.7.1. Single exposures

There have been many estimates of inhalational toxicity in which the theoretical relationship between concentration, duration of exposure, and lethality has been observed. Typical results are LC_{50} values in rats ranging from 31 612 mg/m^3 for a 10-min exposure to 11 620 mg/m^3 for a 60-min exposure. The corresponding value for a 2-h exposure was 7600 mg/m^3. Exposed mice exhibited avoidance behaviour at concentrations above 350 mg/m^3, and ciliary activity was arrested above this level in *in vitro* studies on rabbit tracheal epithelium. Other effects of exposure include bradypnoea and bradycardia, changes in various serum-enzyme levels, and histological changes in the lung. At high concentrations, convulsions occurred.

There have been a number of studies on the oral toxicity of various ammonium salts, some of which have been complicated by the acidity or alkalinity of the preparations used. Median lethal doses for ammonium sulfamate or sulfate were in the range 3 - 4.5 g/kg body weight in both rats and mice. Ammonium chloride causes substantial acidosis and has been reported to produce pulmonary oedema by a different mechanism by gavage, but not by intraperitoneal injection. There is also evidence that ammonium

ions exert a direct effect on the appetite by their effect on prepyriform cortical areas. Ammonium chloride, even after administration for periods of a few days, produces hypertrophy of the kidney, but the extent to which this results from acidosis, a solute load, or a direct effect of the ammonium ion is not clear. Diet and the clinical condition of the liver are important modulators of ammonia toxicity, and it has been shown that the administration of ornithine, aspartic acid, or adenosine triphosphate (ATP) exerts a protective effect against ammonia toxicity.

No information is available regarding systemic toxicity from single dermal exposures to ammonia or ammonium compounds.

Symptoms after intravenous injection of ammonium salts are characterized by immediate hyperventilation and clonic convulsions, followed by either fatal tonic extensor convulsion or the onset of coma, in which tonic convulsions and death can occur at any time. After 30 - 45 min, surviving animals recover rapidly and completely. After injection, neurological symptoms commenced when the blood-ammonia concentration doubled above basal values. Brain-ammonia levels did not increase until blood levels reached 20 times basal values; at this stage, brain levels suddenly increased to about 100 mg ammonia-nitrogen/kg wet weight. However, immediate increases in brain-ammonia after intravenous injection have also been observed, and it has been suggested that there is no critical blood-ammonia concentration for diffusion of ammonia through the blood-brain barrier. Some workers have demonstrated the induction of ventricular fibrillation of the heart following injections of ammonium salts.

1.7.2. Short-term exposures

Ninety-day inhalation exposures of rats to 127 mg/m^3 and 262 mg/m^3 did not produce any, or only minimal, changes. Continuous exposure to 455 mg/m^3 was fatal for 50 out of 51 rats by the 69th day of exposure. Similar results were obtained in guinea-pigs. The principal pathological findings were eye irritation, corneal opacities, and diffuse lung inflammation. Similar results have been published by a number of authors. Concentration-dependent increases in susceptibility to infection during ammonia exposure have been reported. Blood-ammonia levels increased with inhalational exposure to increasing concentrations of ammonia above 70 mg/m^3, for periods of 1 - 7 days.

Studies on the effects of ingestion of ammonium chloride (10 g/litre drinking-water - about 1 g/kg body weight per day) and ammonium sulfamate (5 g/kg body weight per day for 6 days per week) did not show any significant toxic effects. Cyclical administration of various ammonium salts, at moderate doses, for 3 weeks out of 4 affected the reproductive system of virgin female rabbits. Ammonium salts have been given as a dietary supplement to animals on diets deficient in non-essential amino acids, with resultant increases in weight gain. Ammonium salts can prevent and reduce the weight loss associated with 10% and 20% reduction of the crude protein content of the diet of pigs.

There is no information regarding the systemic effects of short-term dermal exposure.

1.7.3. Skin and eye irritation; sensitization

There is little information on animals to complement the

extensive human experience. In rabbits, ammonia has been shown to penetrate the cornea rapidly and to cause corneal burns. Ammonium persulfate is a recognized skin sensitizer in man. No data on sensitization potential in animal models are available.

1.7.4. Long-term exposure

Inhalation exposure studies did not extend beyond 130 days. A 130-day study demonstrated congestion of parenchymatous organs at 18 weeks, but not at 12 weeks, in guinea-pigs exposed to about 119 mg/m^3 for 6 h/day, 5 days/week. Long-term studies have not been carried out according to modern protocols, and observed effects have mainly been related to changes in acid-base balance.

1.7.5. Reproduction, embryotoxicity, and teratogenicity

There have not been any formal studies based on modern protocols, but studies have been undertaken to investigate the effects of ammonia in hen-houses on the egg-laying performance of intensively reared poultry. No systematic conclusions could be drawn.

1.7.6. Mutagenicity

Ammonium sulfate has been reported non-mutagenic in *Salmonella* and *Saccharomyces* test systems, but mutagenic in *E. coli* at toxic levels and may affect mutagenic responses to other agents. Various workers have described effects on *Drosophila,* which were minimal or achieved only at toxic levels. There is no evidence that ammonia is mutagenic in mammals.

1.7.7. Carcinogenicity

There is no evidence that ammonia is carcinogenic, though it can produce inflammatory lesions of the colon and cellular proliferation, which could increase susceptibility to malignant change. There was no evidence that ammonia was responsible for the increased incidence of tumours with increased dietary protein intake. Ammonia did not either cause tumours or increase the spontaneous incidence of tumours in life-time studies on mice.

1.7.8. Mechanisms of toxicity

Although there are a number of hypotheses, there is no established mechanism for the toxicity of ammonia or ammonium salts.

1.8. Effects on Man

1.8.1. Organoleptic effects

Ammonia can be tasted in water at levels above about 35 mg/litre. Odour thresholds have been variously reported according to the definition used and technique of measurement. Most people can identify ammonia in air at about 35 mg/m^3 and can detect it at about one-tenth of this level.

1.8.2. Clinical, controlled human studies and accidental exposure

Exposure to ammonia in air at a concentration of 280 mg/m^3 produced throat irritation; 1200 mg/m^3 produced cough; 1700 mg/m^3 was life-threatening, and more than 3500 mg/m^3 caused a high mortality. Respiratory symptoms were usually reversible, but chronic bronchitis has been reported to develop. Volunteers

exposed by oro-nasal mask experienced irritation and increased minute volumes. Retention of inspired ammonia decreased progressively to about 24% after about 19 min of exposure. The blood chemistry remained normal. Respiratory indices were insignificantly altered at concentrations up to 98 mg/m^3 (which was tolerable). Other studies have demonstrated a high incidence of symptoms at this level. Irritation occurred at 35 mg/m^3, which was neither discomforting nor painful. Industrial exposure at 88 mg/m^3 was described as "definitely irritating".

Ingestion of ammonia solutions has produced caustic burns of the upper gastrointestinal tract. Ingestion of ammonium chloride produces metabolic acidosis and diuresis and is administered for these effects.

1.8.3. Endogenous ammonia

Ammonia plays a key role in nitrogen metabolism, and its level in the body may be increased as a result, either of in-born errors of metabolism, or, as a result of impaired liver function. The role of hyperammonaemia in causing the encephalopathy associated with the latter is not completely clear, but there is sufficient evidence to indicate a significant contribution.

1.9. Evaluation of the Health Risks for Man and Effects on the Environment

Atmospheric exposure of the general population is toxicologically insignificant. Occupational exposure can give rise to symptoms, particularly in occupations exposed to decaying organic matter. Accidental exposure to ammonia in any of its forms produces irritant or caustic effects.

Exposure to ammonia in the water supply and food is insignificant in comparison with the nitrogen intake through the diet which becomes available as metabolic ammonia.

The most significant effects of ammonia are in the aquatic and terrestrial environments where, as a result of urbanization, industry, and farming and as a result of deposition to sensitive environments, significant toxic effects of ammonia may arise.

1.10. Conclusions

Ammonia does not present a direct threat to man except as a result of accidental exposure, particularly in industry. Farm animals may be adversely affected when reared intensively in closed conditions. Localized effects of point-source emissions of ammonia and of deposition in sensitive environments is a cause of concern.

2. PROPERTIES AND ANALYTICAL METHODS

2.1. Physical and Chemical Properties of Ammonia and Ammonium Compounds

2.1.1. Gaseous and anhydrous liquid ammonia

Ammonia (NH_3) is a colourless gas at atmospheric pressure, which is lighter than air and possesses a strong penetrating odour. Some of the relevant physical properties of ammonia are summarized in Table 1.

The vapour pressure of ammonia gas over pure ammonia liquid can be calculated using the equation (NRC, 1979):

$$\log_{10}P = 9.95028 - 0.003863T - 1473.17/T,$$

where P = partial pressure in mm Hg, and T = temperature at K.

Ammonia may be liquefied under pressure at about 10 atm and is stored and transported in this state.

2.1.2. Aqueous solutions

Ammonia dissolves readily in water where it ionizes to form the ammonium ion.

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

The solubility of ammonia in water is influenced by the atmospheric pressure, temperature, and by dissolved or suspended materials. Solubility values at moderate concentrations and temperatures can be obtained from the graphic (Sherwood, 1925) and tabular (Perry et al., 1963) compilations, and from empirical formulae (Jones, 1973).

The total ammonia content of water is the sum of non-ionized (NH_3) and ionized (NH_4^+) species. Ammonia is readily soluble in aqueous systems (Table 1) and, at the pH of most biological systems, exists predominantly in the ionized form. At low concentrations, the molarity of total dissolved ammonia is given by (Drewes & Hales, 1980):

$$[NH_3] + [NH_4^+] = H[NH_{3(gas)}] + K_bH[NH_{3(gas)}],$$

where $[NH_{3(gas)}]$ is the molar concentration of gas-phase ammonia, K_b is the dissociation constant given by:

$$K_b = \frac{[NH_4^+]\,[OH^-]}{[NH_3]} = 1.774 \times 10^{-5} \text{ (at 25°C)}$$

and H is a Henry's law constant given by (NRC, 1979):

$$\log_{10}H = 1477.8/T - 1.6937$$

Table 1. Physical properties of ammonia[a]

Properties	Values
Boiling point at one atm	-33.42 °C
Melting point	-77.74 °C
Density (liquid) at -33.35 °C and 1 atm	0.6818 gm/cm^2
Density (gas)	0.7714 g/litre
Viscosity at -33 °C	0.254 centipoise
Viscosity at 20 °C	9.821 x 10^9 poise
Refractive index at 25 °C	1.325
Dielectric constant at 25 °C	16.9
Surface tension at 11 °C	23.38 dyn/cm

Specific conductance at -38 °C	1.97×10^{-7} cm^{-1}
Thermal conductivity at 12 °C	5.51×10^{-5} gcal/cm
Vapour pressure at 25 °C	10 atm
Critical temperature	132.45 °C
Critical pressure	112.3 atm
Critical density	0.2362 g/cm^3
Solubility in water, 101 kPa	
at 0 °C	895 g/litre
20 °C	529 g/litre
40 °C	316 g/litre
60 °C	168 g/litre

[a] From: Jones (1973) and Windholz et al., ed. (1976).

The pK_a for the ammonia/ammonium equilibrium can be calculated at all temperatures, T(K), between 0 and 50 °C ($273 < T < 323$) by the equation (Emerson et al., 1975):

$$K_a = [NH_3]\,[H^+]/[NH_4+],$$
$$pK_a = 0.09018 + 2729.92/T$$

Theoretically, the fraction (f) of total ammonia that is non-ionized depends on both water temperature and pH, according to the preceding and the following equations (Emerson et al., 1975):

$$f = 1/[10^{(pK_a-pH)} + 1]$$

Thus, in water at 0 °C and a pH of 6, less than 0.01% of the total ammonia present is in the non-ionized form, whereas, at 30 °C and a pH of 10, 89% of total ammonia is non-ionized.

The above relationship holds in most fresh waters. However, the concentration of non-ionized ammonia will be lower at the higher ionic strengths of very hard fresh waters or saline waters. Using the appropriate activity coefficients, in sea water of ionic strength = 0.7, the above relationship can be restated as follows (API, 1981):

$$f = 1/[10^{(pK_a-pH+0.221)} + 1]$$

At 25 °C, the pK_a can be calculated to be 9.24, from the equation of Emerson et al. (1975). Therefore, at pH 8, and at a temperature of 25 °C, the above equation shows that 3.31% of the total ammonia in sea water exists in the non-ionized form. The corresponding value in fresh water can be calculated to be 5.38%. Thus, at this pH and temperature, sea water with an ionic strength of 0.7 would contain 62% as much non-ionized ammonia as fresh water.

2.1.3. Chemical reactions

Gaseous ammonia is readily adsorbed on certain solids. The

adsorption characteristics of ammonia on metal surfaces are important in its synthesis and other catalytic reactions (Cribb, 1964). Because of the adsorption of ammonia on charcoal, acid-impregnated charcoal masks are used for protection against ammonia gas.

Ammonia can form explosive mixtures with air at atmospheric temperature and pressure, if present in concentrations of 16 - 27% by volume. The products of combustion are mainly nitrogen and water, but small traces of ammonium nitrate (NH_4NO_3) and nitrogen dioxide (NO_2) are also formed.

Another important reaction involving the oxidation of ammonia is its catalytic oxidation to nitric oxide (NO) and nitrous oxide (N_2O) (Miles, 1961; Matasa & Matasa, 1968). This reaction is an important step in the manufacture of nitric acid.

Under normal atmospheric conditions, ammonia does not undergo any primary photochemical reactions at wavelengths greater than 290 nm.

When exposed to radicals or other photochemically excited species, ammonia undergoes secondary decomposition:

$$NH_3 + \text{-}OH \rightarrow \text{-}NH_2 + H_2O$$

$$NH_3 + O \rightarrow \text{-}NH_2 + \text{-}OH$$

Some of these reactions may be important in the balance of atmospheric nitrogen.

Ammonia also undergoes decomposition to nitrogen and hydrogen, when exposed to an electric discharge (Jones, 1973). It reacts with sulfur dioxide gas to form ammonium sulfate in the atmosphere (Kushnir et al., 1970).

Aqueous ammonia can take part in substitution reactions with organic halide, sulfonate, hydroxyl, and nitro compounds, and, in the presence of metallic catalysts, it is used to produce amino acids from keto acids. Ammonia reacts with hypochlorous acid (HOCl) to form monochloramine, dichloramine, or nitrogen trichloride (Morris, 1967; Lietzke, 1978). The formation of these *N*-chloramines depends on the pH, the relative concentrations of hypochlorous acid and NH_3, the reaction time, and the temperature. When pH values are greater than 8, and when the molar ratio of HOCl to NH_3 is 1:1 or less, the monochloramine predominates. At higher Cl_2:NH_3 ratios or, at lower pH values, dichloramine and trichloramine are formed. These, and various organic chloramines, are produced during the chlorination of water containing NH_3 or organic amines. The presence of these chloramines may contribute to the taste and odour of drinking-water, and to various associated health problems (Morris, 1978).

2.1.4. Ammonium compounds

Ammonium compounds comprise a large number of salts, many of which are of industrial importance; ammonium chloride, ammonium nitrate, and ammonium sulfate are produced on a large scale. With the exception of metal complexes, the ammonium salts are very similar in solubility to the salts of the alkali metals, but differ in that they are completely volatilized on heating or ashing.

Ammonium salts undergo slight hydrolysis in aqueous solution. Most dissociate at elevated temperatures to give ammonia and the

protonated anion. The physical and chemical properties of ammonium compounds of environmental importance are discussed below, and some of their physical properties are summarized in Table 2.

Ammonium chloride [NH_4Cl] occurs naturally in volcanic crevices as a sublimation product. When it sublimes, the vapour is completely dissociated into hydrogen chloride and ammonia. Like other ammonium salts of strong acids, the chloride hydrolyses in aqueous solution to lower the pH of the solution. The solid tends to lose ammonia during storage. Aqueous solutions of ammonium chloride have a notable tendency to attack ferrous metal and other metals and alloys, particularly copper, bronze, and brass. Ammonium chloride can be oxidized to nitrosyl chloride and chlorine by strong oxidizing agents, such as nitric acid.

Ammonium nitrate [NH_4NO_3] does not occur in nature. It is soluble in water and liquid ammonia and slightly soluble in absolute ethyl alcohol, methanol, and acetone. Although ammonium salts of strong acids generally tend to lose ammonia during storage, ammonium nitrate can be considered a very stable salt. It undergoes decomposition at elevated temperatures or under extreme shock, as in commercial explosives. Ammonium nitrate acts as an oxidizing agent in many reactions, and, in aqueous solution, it is reduced by various metals. Solutions of ammonium nitrate attack metals, particularly copper and its alloys.

Ammonium sulfate [$(NH_4)_2SO_4$] is found naturally in volcanic craters. It is soluble in water and insoluble in alcohol and acetone. The melting point of ammonium sulfate is 230 °C. On heating in an open system, the compound begins to decompose at 100 °C, yielding ammonium bisulfate (NH_4HSO_4) that has a melting point of 146.9 °C.

Ammonium acetate [CH_3COONH_4] is a deliquescent material that is highly soluble in cold water and in alcohol. Solubility does not increase greatly with increasing temperature, at least up to 25 °C. In aqueous solution at atmospheric pressures, ammonium acetate readily loses ammonia, especially in alkaline conditions.

Ammonium carbonate [$(NH_4)_2CO_3$] and ammonium bi-carbonate [NH_4HCO_3] have long been known because of their occurrence in association with animal wastes. Ammonium bicarbonate is the more readily formed and the more stable. It decomposes below its melting point (35 °C), dissociating into ammonia, carbon dioxide, and water. Ammonium bicarbonate reacts with, and dissolves, calcium sulfate scale. Ammonium carbonate decomposes on exposure to air with the loss of ammonia and carbon dioxide, becoming white and powdery and converting into ammonium bicarbonate. Ammonium carbonate volatilizes at about 60 °C. It dissolves slowly in water at 20 °C, but decomposes in hot water.

2.2. Sampling and Analytical Methods

2.2.1. Air and water samples

Measurement of ammonia levels in air is difficult. Atmospheric levels are low, and samples can be contaminated by emissions from man; thus, the analyst should remain remote from the sampling device. In addition, air samples are bubbled through acid media to form an aqueous solution of ammonia, predominately in its ionic form. The extraction of ammonia is variable and both gaseous ammonia and that contained in aerosols will be extracted. In some

instances filters are used to remove aerosols from the gas stream so that only ammonia gas is sampled. There are, however, some problems with aerosol filters as they may interact with gaseous ammonia when the aerosol is collected on the filter (NRC 1979).

Table 2. Physical properties of some ammonium compounds[a]

Property	Ammonium chloride	Ammonium nitrate	Ammonium sulfate	Ammonium acetate	Ammon carbo
Synonyms	ammonium chloride; sal ammoniac	ammonium nitrate	ammonium sulfate; mascagnite	ammonium acetate	ammon carbo monoh
Colour	colourless	colourless	colourless	white	colou
Physical state (25 °C, 1 atm)	cubic crystals	rhombic crystals	rhombic crystals	crystals, hygroscopic	cubic cryst
Formula	NH_4Cl	NH_4NO_3	$(NH_4)_2SO_4$	CH_3COONH_4	$(NH_4)_2CO_3$
Relative molecular mass	53.49	80.04	132.14	77.08	114.1
Melting point (°C)	340 sublimes	169.6	230 decomposes	114	58 decom
Boiling point (°C)	520	> 210 decomposes		decomposes	
Density	1.527 (20 °C)	1.725 (25 °C)	1.769 (50 °C)	1.17 (20 °C)	
Refractive index, n_b^{20}	1.642		1.533		
Solubility in water (g/litre)	370 (20 °C)	1920 (20 °C)	754 (20 °C)	1480 (4 °C)	1000

[a] From: Dean (1979) and Weast (1979).

Air samples collected by liquid impinger yield aqueous solutions. Fabric filters used for collecting aerosols may be extracted with water for analysis. Generally, air and water samples are analysed using similar techniques, which are summarized in Table 3.

Various methods for preventing interference can be used, but distillation at pH 9.5 is often carried out. Care must be taken with water samples to prevent oxidation, volatilization, or microbiological assimilation of ammonia. Thus, samples should be acidified and refrigerated in sealed containers (and may be treated with reagents) and analysed within 24 h (APHA, 1976; NRC, 1979; US EPA, 1979b; ASTM, 1980; API, 1981; Analytical Quality Control (Harmonised Monitoring) Committee, 1982).

2.2.2. Soil samples

Soil samples are usually collected by the grab method. To inhibit microbial activity during transport and storage, reagents (e.g., mercury (II) chloride) can be added to the soil (NRC, 1979). Rapid drying at 55 °C, then sealing the samples in air-tight containers is a more satisfactory method of preservation for

ammonium determination (NRC, 1979), but even this may not prevent erroneous results, and samples should be analysed soon after sample collection (NRC, 1979). Analytical methods for the determination of ammonia and ammonium in soils have been reviewed by NRC (1979).

2.2.3. Blood and tissue samples

The various techniques used for the determination of ammonia in blood and tissues ultimately incorporate the ammonia detection methods described in Table 3, but with various conditions, such as distillation, aeration, and diffusion to minimize interference (NRC, 1979). Because of the higher protein concentration in tissues, determination of ammonia is subject to greater glutamine-caused error than in body fluids (NRC, 1979).

Table 3. Ammonia detection methods

Medium	Particular application	Method	Principle	Interferant
Air	silo air NH_3	alkalimetric	air is drawn through sulfuric acid until bromophenol indicator changes colour; volume of air is inversely proportional to ammonia concentration	other acidic alkaline contaminant
Water	high concentrations	titrimetric	NH_3 in water is distilled off into distilled water which is titrated with acid to a methyl red/ methylene blue end-point	
Air Water		Nesslerization	NH_3/NH_4 in dilute sulfuric or boric acid is reacted with alkaline mercuric and potassium iodide solution ($Hg\ I_2$ x KI); absorbence at 440 nm is compared with a standard curve; distil-lation can preceed analysis	amines, cyan alcohols, a ketones, co turbidity, chlorine
Air	low concentrations	indophenol reaction	NH_3 in solution is reacted with hypochlorite and phenol (slow-warm reagents)	monoalkyl am formaldehyd
Water				turbidity, salt (sea w
Air	measurement of tobacco smoke	ammonia electrode (potentiometric)	measurement of ionization potential of NH_3 --> NH^+_4	mercury, vo amines

Table 3. (contd.)

Medium	Particular application	Method	Principle	Interferant

Water			
Air	continuous measurement	chemiluminescent	air is passed through high- and low-temperature catalytic converters, which respectively measure NO_x + NH_3 and NO_x; NH_3 is obtained by subtraction
Air	tobacco smoke	gas chromato-graphy	gas chromatography with thermal conductivity detector
Air	continuous measurement	UV spectro-photometry	NH_3(gas) exhibits several strong absorption bonds between 190 and 230 nm; absorption in 10 cm quartz cells at 204.3 nm has been used (molecular extinction coefficient = 2790)
Air	continuous measurement high sensitivity	Fluorescent derivatization technique	1-phthaldehyde derivatization

3. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

Ammonia is present in the environment as a result of natural processes and through the industrial activities of man. It is generally accepted that, of the ammonia present in the atmosphere, 99% is produced by natural biological processes. Ammonia is continually released throughout the biosphere by the breakdown or decomposition of organic waste matter. Thus, any natural or industrial process that concentrates and makes nitrogen-containing organic matter available for decomposition represents a potential source of high local concentrations of ammonia in water, air, and soil. Industrially-produced ammonia, from non-biological nitrogen, also represents an environmental source, by release through agricultural fertilization and industrial emissions. Coal gasification or liquefaction may provide a major local source of ammonia. The natural occurrence of ammonia compounds is indicated in section 2.1.4.

3.1. Production and Use

Ammonia is one of the most widely-used industrial chemicals. It is ranked fourth in production volume in the USA after sulfuric acid, lime, and oxygen (Chemical and Engineering News, 1980). Total production of ammonia-nitrogen in the USA increased from 5.8 x 10^6 tonnes in 1964 to 11.5 x 10^6 tonnes in 1974 (Keyes, 1975), and had further increased to 17.6 x 10^6 tonnes by 1979 (Chemical and Engineering News, 1980). The demand in the USA for the production of ammonia is projected to reach 25 x 10^6 tonnes by 1990 (Mai, 1977).

Ammonia is mainly produced industrially by the Haber-Bosch process in which nitrogen and hydrogen are combined under high

pressure in the presence of a catalyst (Harding, 1959; Matasa & Matasa, 1968). Prior to the Haber-Bosch process, ammonia was produced by the hydrolysis of cyanamides or cyanides. A smaller scale method for ammonia production is regeneration from ammonium salts by heating with a base. Alkaline earth metal oxides and hydroxides have been used with the naturally-occurring ammonium chloride.

Most of the ammonia produced in the USA is consumed as fertilizers (80%), fibres and plastics (10%), and explosives (5%) (Chemical and Engineering News, 1980). It is also used in the production of animal feed (1.5%), pulp and paper (0.6%), and rubber (0.5%) (Keyes, 1975) and in a variety of other chemical production processes. Ammonia and ammonium compounds are used as cleaning fluids, scale-removing agents, and in food as leavening agents, stabilizers, and for flavouring purposes. A survey by the US Food and Drug Administration (US FDA) indicated that about 6000 tonnes of ammonium compounds were used in food in 1970 (FASEB, 1974) comprising ammonium bicarbonate, 317 tonnes; ammonium carbonate, 24 tonnes; ammonium hydroxide, 535 tonnes; monobasic ammonium phosphate, 52 tonnes; dibasic ammonium phosphate, 434 tonnes; and ammonium sulfate, 1468 tonnes. Information for ammonium chloride was not available. The use of ammonium compounds in food nearly doubled during the period 1960 - 70.

3.2. Sources Releasing Ammonia into the Air

Ammonia is released into the atmosphere by agricultural, waste-disposal, and industrial activities. Ammonia global release has been estimated at 113 - 244 x 10^6 tonnes ammonia-nitrogen/year (Söderlund & Svensson, 1976). In the USA, industrial emissions from ammonia and fertilizer production (anhydrous ammonia, aqueous ammonia, ammonium nitrate, ammonium phosphates, urea), from petroleum refineries, coke ovens, and sodium carbonate manufacture, and loss of anhydrous ammonia during distribution, handling, and application have been estimated to be approximately 328 x 10^3 tonnes, annually (NRC, 1979; US EPA, 1981). This figure does not include volatilization of ammonia after soil applications of nitrogen fertilizer, which may amount to 5 - 10% of the ammonia and urea fertilizer applied. These losses were estimated to comprise another 285 x 10^3 tonnes, annually (US EPA, 1981).

Combustion processes release ammonia as a by-product in amounts that are dependent on the substance being burned and the conditions of combustion. Assuming that 2% of the municipal wastes generated in the USA are incinerated, about 0.8 x 10^3 tonnes of ammonia would be emitted annually from this source. On the other hand, fossil fuel combustion in the USA is estimated to release 783 x 10^3 tonnes/year (US EPA, 1981).

On the basis of the number of cattle in the USA and an average excretion of 31 kg urea per animal per year, it has been estimated that 3400 x 10^3 tonnes/year of ammonia are produced by cattle in the USA (API, 1981). Similar calculations made in the Netherlands on the basis of the manure production of cattle, pigs, and poultry give a figure of 114 x 10^3 tonnes/year (Buysman, 1984). Estimates of atmospheric emissions from the Netherlands and the USA are shown in Table 4.

It must be emphasized that substantial uncertainties are associated with these estimates, which are given for rough comparison only. Ammonia from sources that cannot be quantified includes that which volatilizes from livestock wastes or polluted

water, and emissions from the combustion of wood. These sources must be considered in perspective with natural sources, especially the microbial fixation of nitrogen and the mineralization of nitrogenous organic matter. Emissions from these natural sources far outweigh those from man-made sources, on a global scale; however, man-made sources can result in locally elevated atmospheric concentrations.

Table 4. Estimated atmospheric emissions of ammonia in the USA and the Netherlands

Source	Annual emission (10^3 tonnes NH_3)	
	USA[a]	the Netherlands[b]
animal manure	3400	114 (1)
fertilizer volatilization	285	6.1 - 10.6
industrial activities	1111	7.6
other sources	0.8	0.5

[a] From: US EPA (1981).
[b] From: Buysman (1984).

The very high contribution to ammonia emission from animal manure production in the Netherlands is remarkable. More than 80% of the annually emitted ammonia results from the production of manure on intensive livestock farms and its use as an agricultural fertilizer. In all areas with intensive livestock farming, ammonia emission from animal manure production contributes 90 - 99% to the total NH_3 emission. The number of poultry and pigs used in livestock farms increased 3 - 5 times between 1950 and 1980, and it can be expected that the ammonia emission from animal manure production has increased similarly. In only a few areas does most of the emitted ammonia result from industrial activities, such as the production of coke and fertilizers, and the combustion of fossil fuel.

In Denmark, Belgium, and some parts of the Federal Republic of Germany and France, animal manure production contributes significantly to atmospheric emissions of NH_3 (Buysman et al., 1985).

3.3. Sources Discharging Ammonia into Water

Ammonia is released into the aquatic environment from a variety of man-made point source discharges and from natural and man-made non-point sources.

3.3.1. Point sources of ammonia

Major man-made point sources discharging ammonia into surface waters include sewage treatment plants, and plants producing fertilizers, steel, petroleum, leather, inorganic chemicals, non-ferrous metals, and ferroalloys, and meat processing plants. Amounts of ammonia discharged annually by these industries in the USA were estimated to be nearly 5.6×10^5 tonnes (API, 1981) (Table 5). These estimates show that the industries examined contribute < 5% of the total ammonia discharged into surface

waters while publicly owned sewage treatment plants (POTWs)

contribute > 95% of the total. It is important to note that the POTW figure is based on an estimated actual discharge, while several of the industrial figures are based on Best Practicable Control Technology (BPT) guidelines and other industrial data.

An estimate of ammonia discharge by sewage treatment plants was based on an average ammonia concentration of 15 mg/litre in secondary treatment waste waters (Metcalf & Eddy, Inc., 1972) and a total discharge of 104 billion litres per day. However, some sewage treatment plants discharge waste waters containing much higher ammonia concentrations. Using data from Mearns (1981), the US EPA (1981) estimated that the mean effluent concentration of ammonia from 5 major POTWs in southern California was 107 mg NH_3-N/litre (130 mg NH_3/litre).

The iron and steel industries release ammonia, as a by-product of the conversion of coal to coke, and during blast furnace operations. The source estimate in Table 5 is based on proposed BPT effluent control limits and steel production data.

The estimated ammonia contribution from the fertilizer industry was based on 1978 production figures for ammonia, ammonium nitrate, urea solutions, and urea solids, and on BPT guideline limits. This contribution may be underestimated because relatively few of these producers meet BPT limits and because production is increasing (US EPA, 1981).

The estimated contribution of ammonia for all other industry groups in Table 5, except the meat processing and leather industries, was based on production figures and BPT guideline limits. The effluents of the meat processing and leather industries were reported to contain about 40 and 100 mg NH_3/litre, respectively (API, 1981).

3.3.2. Non-point sources of ammonia

Non-point sources of ammonia for surface waters are not as easy to quantify as point sources. Non-point sources include releases not discharged by a discrete conveyance. They are variable, discontinuous, diffuse, and differ according to specific land use. They may be the result of runoff from urban, agricultural, silvicultural, or mined lands. Urban runoff may sometimes be considered a point source, as it is frequently collected and discharged from drainage systems. Several hydrological models are available to predict runoff and estimate pollutant loading, but there is still difficulty with this subject. Major non-point sources of ammonia for surface waters include fertilizer runoff, animal feedlots, animal wastes spread on the soil, urban runoff, and precipitation.

Table 5. Estimates of aquatic emissions of ammonia[a] from point sources in the USA

Point source	Estimated contribution (tonnes NH_3-N/year)
Sewage treatment plants (POTWs)	535 922.3[b]
Steel industry	12 951.0[c]
Fertilizer industry	5955.9[c]

Petroleum industry	2826.1[c] or 2767.2[b]
Meat processing industry	1099.3[b]
Leather industry	687.1[b]
Inorganic chemicals industry	99.8[c]
Non-ferrous metals manufacturing	0.9[c]
Ferroalloy manufacturing industry	0.3[c]
Total	559 542.7 tonnes/year

[a] Adapted from: API (1981).
[b] Estimated contribution based on reported or estimated actual discharge concentrations.
[c] Estimated contribution based on production data and BPT guidelines, not actual discharges.

The ammonia content of urban runoff is variable, depending, in part, on specific land use. In a study of urban runoff, the amount of ammonia present varied with the seasons of the year. Ammonia concentrations ranged from 0.18 mg N/litre in the autumn to 1.4 mg N/litre in the early spring (Kluesener & Lee, 1974). In another study, Struzewski (1971) reported that ammonia-nitrogen in urban storm water ranged from 0.1 to 2.5 mg/litre.

The ammonia content of rural runoff originates from natural and man-made sources, including wastes from wildlife and livestock, decaying vegetation, fertilizer applications, material originally present in the soil, and precipitation. Estimating total rural runoff quantities and ammonia concentrations is extremely complex and no overall estimates are available. Loehr (1974) reported that the ammonium-nitrogen concentrations in the drainage from 4 forested watersheds ranged from 0.03 to 0.08 mg/litre. The ammonium-nitrogen concentrations were 8 - 14% of the nitrate-nitrogen concentrations.

Precipitation is also a significant non-point source of ammonia. Concentrations may vary locally, reflecting local atmospheric sources. The average concentration in rainfall at one rural location on Long Island, New York (0.18 mg NH_3-N/litre) was less than half those at 2 other Long Island sites closer to the New York urban area (0.43 and 0.459 mg NH_3-N/litre) (Frizzola & Baier, 1975). Among collection sites throughout Wisconsin, ammonia levels in urban rain samples differed little from those in rural samples not taken near barnyards (range, 0 - 3 mg NH_3-N/litre); however, values for locations near barnyards were 4 - 5 times higher (range 0 - 3 mg NH_3-N/litre) indicating contamination from locally-generated atmospheric ammonia (Hoeft et al., 1972).

Similar tendencies have been observed in the Netherlands, though the absolute data are much higher. In agricultural areas with dense livestock farming, ammonia levels ranging from 2.9 mg NH_4+-N/litre near slurry manured croplands to 5.4 mg NH_4+-N/litre at a distance of 100 m from a poultry farm have been found. In relatively unaffected areas along the northern coast, the average value was 1.2 mg NH_4+-N/litre, because of the relatively high background levels of atmospheric ammonia. The average concentration in wet deposition was 2.4 mg NH_4+-N/litre (Schuurkes, in press).

Watershed studies from pristine forests (Fisher et al., 1968), rural wood and pasture lands (Taylor et al., 1971), and heavily fertilized crop lands (Schuman & Burwell, 1974) have all shown that rainfall nitrogen, including ammonia-nitrogen, accounts for a substantial proportion (50 - 100%) of nitrogen in surface runoff.

3.3.3. Comparison between point and non-point sources

Little information is available for the accurate comparison of point and non-point sources of ammonia for surface waters. In one study, Wilkin & Flemal (1980) examined 3 Illinois river basins to determine the relative sources of various pollution loadings (by mass balance accounting) and the possible extent of water quality improvement by controlling various types of sources. The three river basins, showed differences in point sources, patterns of land use (which influences non-point sources), and ammonia-nitrogen concentrations. The east DuPage basin (42% industrial and urban) contained 4.73 mg NH_3-N/litre, the upper Sangamon basin (19% urban and industrial), 2.51 NH_3-N/litre, and the west DuPage basin (2% urban and industrial), 0.22 mg NH_3-N/litre. The fraction of ammonia-nitrogen load from undefined (non-point) sources in the heavily-industrialized east branch DuPage was only 0.54 compared with 0.84 in the rural upper Sangamon. The authors concluded that much of the pollution loading appeared to be related to undefined sources and that further restrictions on point-source contributions might not result in improved water quality.

These data indicate that, although point sources contribute a large fraction of ammonia loading to surface waters, the contribution of undefined non-point sources is also significant.

4. ENVIRONMENTAL TRANSPORT, DISTRIBUTION, AND TRANSFORMATION

Ammonia in the environment is a part of the total biotic and abiotic nitrogen balance as represented by the nitrogen cycle. The processes of the nitrogen cycle consist of nitrogen fixation, assimilation, ammonification, nitrification, and denitrification. Nitrogen fixation and ammonification are microbially-mediated processes that produce ammonium ions from nitrogen gas and organic nitrogen. Assimilation is the uptake and incorporation of inorganic nitrogen into organic molecules by microbes and plants. Nitrification is the microbial oxidation of the ammonium ion to nitrite (NO_2^-) and nitrate (NO_3^-). Denitrification converts nitrate to nitrogen gas or nitrous oxide.

4.1. Uptake and Transformation in Atmosphere

Ammonia enters the atmosphere as a result of both natural and artificial processes on the Earth's surface; there is no known photochemical reaction by which ammonia could be produced in the atmosphere (NRC, 1979). Atmospheric ammonia undergoes four main types of reaction, namely aqueous-phase reactions, thermal reactions, photochemical reactions, and heterogeneous reactions.

In the aqueous-phase reactions, oxidation of aqueous sulfur dioxide in the presence of ammonia results in the formation of atmospheric ammonium sulfate aerosols. This process is favoured by high humidity, high ammonia concentrations, and low temperatures (NRC, 1979).

Thermal reactions involving anhydrous ammonia and sulfur dioxide may, via heteromolecular nucleation, also result in the formation of ammonium sulfate aerosols. Thermal reactions of

ammonia with ozone result in the formation of ammonium nitrate, but the importance of this mechanism in the production of atmospheric ammonium nitrate aerosols is not known (NRC, 1979).

Photolytic degradation and reaction with photolytically produced hydroxyl radicals (-OH) in the troposphere are major pathways for the removal of atmospheric ammonia. While there is limited information on the relative importance of these different reactions, it has been suggested that one-half of the atmospheric ammonia may be destroyed by the reaction with hydroxyl radicals, with the balance being destroyed by reaction with soot particles or by deposition (wet and dry) as particulate ammonium (NRC, 1979).

In addition to the formation of ammonium sulfate and nitrate, various ammonium surface complexes may be formed by the heterogeneous reaction of atmospheric ammonia with nitric oxide-soot surfaces in the atmosphere (NRC, 1979). While these heterogeneous reactions are significant in combustion reactions, their importance in the atmosphere at much lower concentrations of both ammonia and soot particles, is not known (NRC, 1979).

Comparison of the findings of Robinson & Robbins (1971) and Söderlund & Svensson (1976) on global nitrogen balances for ammonia reveals differences and it is difficult to evaluate which is the more accurate.

4.2. Transport to the Earth's Surface

Most of the ammonia entering the atmosphere will be transported back to the earth by both wet and dry deposition. Wet deposition includes rainfall, snow, hail, fog, and dew, while dry deposition mainly concerns gaseous ammonia. In the Netherlands, a comparison has been made between the total emission and total deposition of ammonia- and ammonium-nitrogen. Almost 95% of the emitted ammonia (119×10^3 tonnes/year) is deposited back on the surface (van Aalst, 1984). In this way, ammonia contributes 60 - 90% of the nitrogen loading of water and soil, with nitrogen oxides making up the other part.

4.2.1. Wet and dry deposition

A part of the ammonia in the atmosphere is removed by washout and rainout. Ammonium sulfate aerosols are produced by aqueous-phase reactions. Thus, wet deposition of ammonia can be estimated by measuring ammonium concentrations in precipitation. Annual average concentrations in wet deposition at locations in 21 European countries vary from 0.12 to 1.74 mg NH_4+-N/litre (Fuhrer, 1985). In the Netherlands, the mean annual concentration for the period 1978 - 82 was 2.4 mg NH_4+-N/litre, corresponding to a wet deposition of 12.2 kg/ha per year. The wet deposition in Norway ranges from 1.3 kg/ha per year in the centre of the country to 8.6 kg/ha per year in the south (calculated from Overrein et al., 1980). In the United Kingdom, it varies between 3.2 and 6.0 kg/ha per year (calculated from Warren Spring Laboratory, 1982).

A comparison has been made of the amounts of dry and wet deposition of ammonia and ammonium per area in the Netherlands. The data are summarized in Table 6.

Wet deposition plays only a minor part (1/3) in the total deposition of NH_3 and NH_4+. On average, 28.4 kg NH_3 + NH_4+-N is deposited per ha per year. However, in rural areas with dense livestock farming, values may reach up to 50 - 100 kg per year.

Table 6. Dry and wet deposition of $NH_3 + NH^+_4$ per ha per year in the Netherlands[a]

	mol/ha per year	kg/ha per year
dry deposition	1150	16.2
wet deposition	790	12.2
Total	1940	28.4

[a] From: Van Aalst (1984).

4.2.2. Contribution to acid rain

Although ammonia is a base and thus increases the pH of rain water, it contributes to the acidifying action of deposition. In particular, the conversion of ammonium to nitrate appears to be important in the acidification of soil and water in carbonate-poor environments (Roelofs, in press; Schuurkes, in press). This potentially-acidifying action has been implicated in the acid rain problem in the Netherlands. On average, NH_3 contributes about 32% of the total deposition of potentially-acidifying substances (Table 7).

Table 7. Average deposition of acid and acidifying substances (acid eq.,/ha per year) in the Netherlands[a]

	SO_2	NO_x	NH_3
Total deposition	2750 (46%)	1310 (22%)	1940 (32%)

[a] From: The Netherlands Ministry of Housing, Physical Planning, and Environment (1984).

4.3. Transformation in Surface Water

Nitrification is important in preventing the persistence or accumulation of high ammonia levels in waters receiving sewage effluent or runoff. The overall reaction is:

$$NH_4+ + SO_2 \longrightarrow 2H^+ + NO_3^- + H_2O$$

It occurs in two steps, involving primarily two bacterial genera, and forming nitrite as an intermediate.

$$NH_4+ \xrightarrow{\text{Nitrosomonas}} NO_2^-$$

$$NO_2^- \xrightarrow{\text{Nitrobacter}} NO_3^-$$

The process depends on many factors, including the amount of dissolved oxygen, temperature, pH, the microbial population, and the nitrogen forms present. Nitrification is an oxygen-consuming process, requiring 2 moles of O_2 per mole of NH_4+ consumed and yielding hydrogen ion (NRC, 1979). Nitrification may thus lead to a depletion of dissolved oxygen and acidification, which may, in turn, inhibit microbiological nitrification (Knowles et al., 1965; Schuurkes et al., 1985).

Anthonisen et al. (1976) reported that, at high levels of total ammonia and a high pH, the resulting concentrations of free ammonia were toxic to both nitrifying forms, but especially to nitrobacters, occasionally leading to the accumulation of nitrite.

Other authors (Kholdebarin & Oertli, 1977) have reported that high pH alone, in the absence of ammonia, can inhibit nitrite oxidation. At high nitrite levels, formation of free nitrous acid caused inhibition of nitrosomonad bacteria, resulting in the persistence of both ammonia and nitrite. However, inhibitory conditions and persistence of reduced forms are usually transient (Anthonisen et al., 1976), and reports of high nitrite levels are rare (Ecological Analysts, Inc., 1981).

Other mechanisms also act to remove ammonia from natural waters. Ammonia is assimilated by aquatic algae and macrophytes for use as a nitrogen source. Ammonia in water may be transferred to sediments by adsorption on particulates, or to the atmosphere by volatilization at the air-water interface. Both processes have been described as having measurable effects on ammonia levels in water; however, the relative significance of each will vary according to specific environmental conditions (API, 1981).

4.4. Uptake and Transformation in Soils

Ammonia levels in soils are a function of the balance between natural and man-made activities. As a result of aerobic degradation processes, ammonia is the first inorganic nitrogenous compound to be released from organic matter together with amines, which are rapidly converted to ammonia (Powers et al., 1977). Other important sources of ammonia in soil are fertilizers (primarily anhydrous ammonia, ammonium nitrate, and urea, which is rapidly converted to ammonia), wet and dry deposition, and animal wastes.

The ammonium cation is relatively immobile in soils, because it is adsorbed on the negatively-charged clay colloids present in all soils (Wallingford, 1977). Ammonia may be lost from soils by volatilization, especially after the application of ammonia fertilizers (Walsh, 1977), sewage, or manures, and by uptake of ammonium ions into root systems. However, the most likely fate of ammonium ions in soils is conversion to nitrate by nitrification. Nitrate is, in turn, lost from soils by: leaching, which occurs readily, since it is repulsed by the clay particles; denitrification, which occurs rapidly within a few days or weeks in warm, moist soils; and by uptake by the plant root system.

5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

5.1. Environmental Levels

5.1.1. Atmospheric levels

Ammonia is present in the atmosphere in very low concentrations, which vary with underlying land use. In most situations, urban atmospheres contain more than non-urban, but certain rural areas, for example, those characterized by intensive animal husbandry or use of organic manure, have atmospheric ammonia levels that exceed urban values. Atmospheric ammonia levels also show a seasonal variation, the highest levels being attained during the winter and the lowest during the summer months. In urban areas, the ammonia levels may increase substantially during pollution episodes. However, they do not show any circadian

patterns.

Urban and non-urban atmospheric levels of ammonia at some locations around the world are shown in Tables 8 and 9. It can be seen that ammonia levels of 4 - 5 μg/m^3 and 20 μg/m^3 are typical of non-urban and urban sites, respectively. Levels of particulate NH_4+ ions in the atmosphere above the main oceans (Atlantic, Pacific, Indian, and Antartic) have been studied; in the southern hemisphere, remote from terrestrial sources, the NH_4+ concentrations were found to be between 10 and 115 ng/m^3. The authors concluded that the oceans are a source of ammonia for the atmosphere (Servant & Delaporte, 1983).

Atmospheric levels of particulate ammonium at some non-urban and urban locations around the world are shown in Table 10. It can be seen that concentrations of 1 μg/m^3 and 4 - 5 μg/m^3 are typical for non-urban and urban sites, respectively.

5.1.2. Levels in water

The concentration of ammonia in surface waters varies regionally and seasonally. Wolaver (1972) studied US Geological Survey data for total ammonia and reported average concentrations of < 0.18 mg/litre in most surface waters, and around 0.5 mg/litre in waters near large metropolitan areas. Analysis of data from the Water Quality Control Information (STORET) System for the years 1972 - 77 (US EPA, 1979a) showed that, although total ammonia-nitrogen concentrations in surface waters in the USA tended to be slightly lower during summer months than during winter months, the percentage of areas in which non-ionized ammonia concentrations occasionally exceeded 0.02 mg/litre increased from 11% during winter to 23% during summer; these percentages were higher when waters had elevated pH values.

Table 8. Urban and industrial atmospheric levels of ammonia in a few global locations[a]

Location	Year	Concentration (μg/m^3)	Reference
Germany, Federal Republic of			
Frankfurt-am-Main	pre-1963	8 - 20	Georgii (1963)
Italy			
Cagliari	-	37 - 280 (highest conc. in the vicinity of port)	Spinazzola et al. (1966)
Japan			
Tokyo	-	up to 210 (down-wind from two major pharmaceutical plants)	TMRI (1971)
Tokyo	1969	4.8 - 25.8	Okita & Kanamori (1971)
Tsuruga	-	up to 6.8	FEPCC (1972)
Netherlands			
Bilthoven	1983	5	Van Aalst (1984)
Delft	1979-81	4.4	Van Aalst

			(1984)
USA			
Seattle, Washington	1975	0.8 - 77.0	Farber & Rossano (1975)
St. Louis, Missouri	1972-73	up to 17.5	Breeding et al. (1976)
Five urban sites in California	-	3 - 60 (average 20.0)	Hidy (1974)
Chino-Corona area, California	1975	up to 315 (vicinity of dairy farm)	Pitts & Grosjean (1976)
USSR			
Environment of metallurgical plant	1967	190	Saifutdinov (1966)
West Berlin	-	up to 97 (average 17.6)	Hantzsch & Lahmann (1970)

[a] Adapted from: NRC (1979). Industrial activities include intensive farming activities.

Table 9. Non-urban atmospheric levels of ammonia in a few global locations[a]

Location	Year	Concentration ($\mu g/m^3$)	Reference
Harwell, England	1969	up to 5.1 (typical level 0.85 - 1.7)	Healy et al. (1970)
Maritime stations (North Sea, Italian coast, and Hawaii)	pre-1963	2 - 5	Georgii (1963)
Rural and mountain locations in Switzerland and the Federal Republic of Germany	pre-1963	5 - 8	Georgii (1963)
Non-urban locations	-	4 - 5	Robinson & Robbins (1968); McKay (1969)
Rural sites in USA	1971	1.4 - 4.2	Breeding et al. (1973)
Boulder, Colorado, USA	1975	2.0 - 3.1	Axelrod & Greenberg (1976)
American tropic	1967-68	3.5 - 21.7 (average 10.5)	Lodge et al. (1974)
Non-urban sites in California, USA	1972	4.6 - 9.7	Hidy (1974)

[a] Adapted from: NRC (1979).

In the Netherlands, enhanced ammonium levels are also present in waters that are not influenced by surface run-off. In particular, in hydrologically-isolated acidified small lakes, concentrations may reach up to 3 mg NH_4+-N/litre. In rural areas with high atmospheric ammonia levels, the loading of these small

lakes with airborne ammonia substances appears to be responsible. The highest measured value near intensive pig and poultry farms was 12 mg NH_4+-N/litre (Leuven & Schuurkes, 1984).

There are few data on the concentrations of ammonia in drinking-water. This is possibly because of the conversion of most of the available ammonia to N-chloramines (mono-, di-, and tri-chloramines) during the chlorination of drinking-water (Morris, 1978), which reduces ammonia concentrations to levels below analytical detectability. The presence of these N-chloramines may contribute to the taste, odour, and also the potential health problems of drinking-water.

Table 10. Urban and non-urban atmospheric levels of particulate ammonium in some global locations[a]

Location	Year	Concentration ($\mu g/m^3$)	Reference
Non-urban:			
England			
Harwell	-	3 - 4	Healy (1974)
(troposphere)	1971-73	1.3	Reiter et al. (1976)
Germany, Federal Republic of			
Bavaria (lower troposphere)	-	1.0	Georgii & Muller (1974)
USA			
28 non-urban sites	1968	0 - 1.2	US EPA (1972)
Point Arguello, California	-	0.36	Hidy (1974)
Goldstone, California	-	0.76	Hidy (1974)
Urban:			
Belgium			
Ghent	1972	1.3 - 33.0 (severe pollution episode)	Demuynck et al. (1976)
Japan			
Nagoya	1973-74	2.7 - 4.2	Kadowaki (1976)
Netherlands			
Delft	1979-81	4.6	Van Aalst (1984)
Terschelling	1982	2.7	Van Aalst (1984
Houtakker	1983	19	Van Aalst (1984)
Sweden			
Rao	-	2.2 - 7.2 (aerosol originating from England)	Brosset et al. (1975)
United Kingdom			
Tees River Valley	1967	up to 33.0 (severe pollution episode)	Eggelton (1969)

Table 10. (contd.)

Location	Year	Concentration ($\mu g/m^3$)	Reference
USA			
Urban areas	1968	0 - 15.1	US EPA (1972)
Five cities	1970-72	0 - 21	Lee & Goranson (1976)
Tuscon, Arizona	1973-74	0 - 6.5	Keesee et al. (1975)
Los Angeles, California	1969-70	2.8 - 3.4	Gordon & Bryan (1973)
15 urban sites in California	-	average 5.3	Hidy (1974)
Riverside, California	1975	up to 30.1 (average 7.6)	Grosjean et al. (1976)

[a] Adapted from: NRC (1979).

Ground water is frequently used as drinking-water, without prior chlorination. Ammonia levels in ground water are usually low because the adsorption of the ammonium ion on clay minerals, or its bacterial oxidation to nitrate, limit its mobility in soil (Feth, 1966; Liebhardt et al., 1979). However, nitrogen fertilizers, livestock wastes, or septic tanks may contribute significant amounts of ammonia to shallow ground waters, especially those underlying poorly-drained soils (Gilliam et al., 1974; Rajagopal, 1978). In domestic tap water from Michigan wells averaging 20 m in depth, mean levels of ammonia-nitrogen were between 0.04 and 0.18 mg/litre; the highest reported single value was 0.57 mg/litre from a well 12.5 m in depth (Rajagopal, 1978).

In wells drilled for research purposes and not supplying drinking-water, levels of ammonia-nitrogen in shallow (3 m) wells beneath wood and crop land usually averaged less than 2 mg/litre (Gilliam et al., 1974). Levels in shallow (3 - 6 m) ground water beneath plots spread with poultry manure varied typically between 1 and 15 mg NH_3-N/litre (Liebhardt et al., 1979); those in ground water beneath 29 feedlots averaged 4.5 mg NH_3-N/litre and ranged up to 38 mg/litre (Stewart et al., 1967). Levels in hot springs and other ground waters have been reported to reach > 1000 mg NH_3-N/litre (Feth, 1966).

The ammonia levels present in the runoff of receiving surface waters have been measured in various studies. Kluesner & Lee (1974) found that levels ranged from approximately 0.23 mg ammonia/litre in the autumn to 1.8 mg ammonia/litre in the early spring in the urban runoff of Madison, Wisconsin. Struzewski (1971) reported that ammonia levels in urban storm water ranged from 0.1 to 3.2 mg/litre.

Only limited data are available on nitrogen pools in the ocean. Söderlund & Svensson (1976) used values of 5 µg NH_3-N/litre for deep areas and 50 µg NH_3-N/litre for near-shore areas and estimated an ammonia inventory of approximately 9×10^3 µg/litre in coastal upwelling systems.

Interstitial water in sediments rich in organic matter contain higher concentrations of ammonia. Sholkovitz (1973) reported values of 1.4 - 23.8 µg ammonia/litre in the interstitial waters of the Santa Barbara Basin. The interstitial water of the Long Island Sound, 2 km off shore, contained concentrations ranging from 11.2 to 42 µg ammonia/litre (Gold-haber & Kaplan, 1974).

5.1.3. Levels in soil

The quantity of ammonia bound to clay in soil has not been estimated. The ammonia present in soil is in dynamic equilibrium with nitrate and other substrates of the nitrogen cycle and is difficult to measure as its concentration is in constant flux (NRC, 1979).

5.1.4. Food

There is very little ammonia in unprocessed food and in drinking-water derived from deep ground-water or chlorinated sources. Various salts of ammonia are added to foods (Annex I).

5.1.5. Other products

Ammonium chloride is a common ingredient in expectorant cough mixtures and is a component of tobacco smoke (about 40 µg/cigarette) (Sloan & Morie, 1974).

5.2. General Population Exposure

Exposure via inhalation and ingestion must be compared to the endogenous production of ammonia in the intestinal tract, which is of the order of several grams per day (section 7.1.2). The relative importance of the different sources is indicated in Table 11.

5.2.1. Inhalation

Assuming ammonia and ammonium concentrations in non-urban and urban air are 2 and 6 µg/m^3 and 24 and 25 µg/m^3, respectively, and that the amount of air breathed per day by an individual is 20 m^3, the intake of total ammonia through inhalation can be calculated to be 0.1 - 0.5 mg/day; the amounts exhaled are considerably higher.

The average amount of ammonia inhaled from the smoking of one cigarette is approximately 42 µg (Sloan & Morie, 1974). Assuming an individual smokes 20 cigarettes per day, the inhalation of ammonia through cigarette smoking would be 0.8 mg/day.

5.2.2. Ingestion from water and food

Most drinking-water in the USA is chlorinated, which effectively eliminates ammonia. However, assuming the direct consumption of 2 litres per day of untreated surface water, at an average total ammonia concentration of 0.18 mg/litre (Wolaver, 1972), the average human uptake from this source would be 0.36 mg per day.

Table 11. Intake of ammonia from different sources

Source	mg/day
Endogenous	4000
Exogenous	
Ingestion (food and drink)	~18
Inhalation	< 1
Cigarette smoking (20/day)	< 1

Although ammonia is a negligible natural constitutent of food, it is formed in the intestine by deamination of the amino groups of food proteins. In addition, ammonium compounds are added in small amounts (< 0.01 - 20 g/kg) to various foods as stabilizers, leavening agents, flavourings, and for other purposes (FASEB, 1974). Information concerning the usual concentrations of ammonium salt additives in foods and the estimated total quantities of these compounds used for this purpose in the USA in 1970 has been used to estimate the average daily intake of 6 ammonium salt additives (FASEB, 1974). The estimates for ammonium bicarbonate, carbonate, hydroxide, monobasic phosphate, dibasic phosphate, and sulfate were 42, 0.3, 7, < 0.1, 6, and 20 mg, respectively. No estimate was available for ammonium chloride. On this basis, the average daily ammonia intake from these compounds has been calculated to be 18 mg.

5.2.3. Dermal exposure

Very few data are available concerning levels of dermal exposure to ammonia or ammonium compounds. Dermal exposure of human beings mainly occurs through the use of household cleaning products, accidental spillage, or under occupational conditions.

5.3. Occupational Exposure

Exposure to ammonia or ammonium compounds can occur in certain occupations involving their production, transportation, and use in agricultural and farm settings, during fertilizer application, or as a result of animal waste decomposition.

It is estimated that about half a million workers in the USA, in a wide variety of occupations, have potential exposure to ammonia (NIOSH, 1974).

Ammonia is generated as a by-product in a wide variety of industrial activities, and workplace atmospheric concentrations are given in Table 12. Municipal waste incineration and gas-fired industrial incinerators generate concentrations of 20 and 0.4 mg/m^3, respectively (NRC, 1979) and shipboard and quayside levels

for natural gas tankers may be about 30 mg/m^3 (Avot et al., 1977). Levels in intensive livestock-rearing buildings are frequently reported to be up to 30 mg/m^3 (Poliak, 1981) or more (Anderson et al., 1964b; Taiganides & White, 1969; Marschang & Petre, 1971). Ammonia levels in dairy farms and cattle-fattening facilities in Romania have been reported to range from 0.7 mg/m^3 to 140 mg/m^3 (Marchang & Crainiceanu, 1971; Marschang & Petre, 1971).

Maximum daily intake from work-place concentrations such as these would normally be less than 300 mg/day and this may be compared with endogenous production (Table 11).

Occupational exposure limits for some countries in the world are shown in Table 13.

5.4. Exposure of Farm Animals

Farm animals are exposed to ammonia through feed containing urea or various ammonium salts and to atmospheric ammonia due to bacterial decomposition and volatilization of ammonia from animal wastes.

5.4.1. Oral exposure

(a) *Non-protein nitrogen additives*

Urea and various ammonium salts have been used for several years as non-protein nitrogen sources in ruminant nutrition. It is used much more widely for this purpose than the ammonium compounds. Urea is hydrolysed to ammonia and carbon dioxide by the ruminal bacteria and, therefore, represents a source of ammonia exposure. The ammonia released is used by the ruminal microorganisms to synthesize microbial protein, which is then digested in the small intestine of the ruminant and used as a source of dietary amino acids.

(b) *Refeeding of livestock wastes*

Results of studies on the refeeding of livestock wastes (Bhattacharya & Taylor, 1975; Arndt et al., 1979; Smith & Wheeler, 1979) have indicated that manure could be of nutritive value, salvaging some nutrients ordinarily lost (Yeck et al., 1975). The non-protein nitrogen (e.g., urea, uric acid) present in livestock wastes is available to ruminants because of microbial conversion in the rumen. Wastes are of limited value for monogastrics such as swine.

Table 12. Ammonia levels in some industrial processes[a]

Operation	Level (mg/m^3)
Machinery manufacturing (cleaning)	10.5
Diazo-reproducing machine	5.6
Mildew-proofing	87.5
Electroplating	38.5
Galvanizing, ammonium chloride flux	7 - 61.6
Blueprint machine	7 - 31.5
Printing machine	0.7 - 31.5
Etching	25.2
Refrigeration equipment	6.3 - 25.9
Cementing insoles	5.6 - 19.6
Chemical mixing	42 - 308
Fabric impregnating	ND

[a] From: NIOSH (1974).
ND = Not detectable.

5.4.2. Inhalation exposure

(a) *Ruminants*

Marschang & Crainiceanu (1971) measured the ammonia concentrations in air (sampled at nose level of animals) in calf stables at 4 dairy farms in Romania. The ammonia levels ranged from 0.7 to 140 mg/m^3 (1 - 200 ppm). Most of the observed values greatly exceeded the permissible upper limit of 18.2 mg/m^3 (26

ppm). In a second study, Marschang & Petre (1971) measured the ammonia concentrations in the air of 3 cattle-fattening facilities in Romania in which the animals were being fed in total confinement; the capacities of the 3 operations were 3000, 3000, and 4900 animals. The ammonia concentration ranged from 2 to 1400 mg/m^3 (3 to 200 ppm). In general, the ammonia content was below the admissible upper limit during the summer months but exceeded it during the winter months, when extremely high concentrations were observed. These high concentrations were primarily due to the blocking of the ventilation system, in order to maintain necessary stall temperatures. The highest value (1400 mg/m^3) was measured when the cleaning mechanism of the manure canals malfunctioned.

Table 13. Occupational exposure limits (mg/m^3)[a]

Country	Ammonia		Ammonia chloride		Ammonium sulfamate	
	A	B	A	B	A	B
Australia	18		10		10	
Belgium	18		10		10	
Czechoslovakia	40	80				
Finland	18					
German Democratic Republic		20		10		
Germany, Federal Republic of	35		10		15	
Hungary	20					
Italy	20				10	
Japan	18					
Netherlands	18		10		10	
Poland	20					
Romania	20	30	5	10	10	15
Sweden	18	36				
Switzerland	18		6		10	
USA (NIOSH/OSHA)	35				15	
(ACGIH)	18	27	10	20	10	20
USSR	20				10	
Yugoslavia	35				15	
Council of Europe	18				15	

[a] From: ILO (1970).
Column A represents average values.
Column B is higher quoted limits, which may variously be ceiling values, short-term exposure limits, etc.

Note: Occupational exposure levels and limits are derived in different ways, possibly using different data, and expressed and applied in accordance with national practices. These aspects should be taken into account when making comparisons.

(b) *Swine*

The increased use of confined housing for swine has caused concern about the purity of the air within the buildings and its effects on swine growth. Bacterial decomposition of excreta collected and stored beneath slotted floors in enclosed buildings produces a number of gases, including ammonia, carbon dioxide, hydrogen sulfide, and methane (Curtis, 1972). Miner & Hazen (1969) reported a range of ammonia concentrations of 4.2 - 24.5 mg/m^3 (6 - 35 ppm) determined 30 cm above the floor level in a swine-rearing facility. Levels in solid-floor confinement units were normally found to be < 35 mg/m^3 (< 50 ppm), but they could be higher during cold months, when ventilation was at a minimum, particularly when the floor was heated (Taiganides & White, 1969). The normal ammonia concentration in the air above slotted floors was reported to be ~7 mg/m^3 (~10 ppm), but this was increased by a factor of 5 - 10 by stirring the stored manure.

(c) *Poultry*

Poultry are usually exposed to ammonia, together with hydrogen sulfide, carbon dioxide, and methane, in the air of poultry houses. These compounds result from bacterial action on poultry wastes (Ringer, 1971). In cold climates, proper ventilation rates cannot be maintained in many poultry houses, and gas production in the manure may build up to toxic levels. Ammonia has been found at concentrations exceeding 35 mg/m^3 (50 ppm) in modern poultry houses, and at up to 140 mg/m^3 (200 ppm) in poorly-ventilated poultry houses (Anderson et al., 1964b; Valentine, 1964). The toxic effects in poultry can be prevented through proper management practices (Lillie, 1970).

6. EFFECTS ON ORGANISMS IN THE ENVIRONMENT

6.1. Microorganisms

Many microorganisms are able to use ammonia as a nitrogen source for cellular nutrition. Nitrifying organisms derive energy from the oxidation of ammonia to nitrate. High levels of ammonia and high pH, which may occur, for example, in waste waters or fertilized fields, may inhibit nitrification and cause persistance or accumulation of ammonia and/or nitrite. Improper maintenance of conditions in waste treatment processes may result in ammonia overloading and inhibition of the nitrification process, with consequent ammonia and/or nitrite pollution of receiving surface waters. Other soil microorganisms may also be inhibited; fungi reportedly are more sensitive than bacteria. However, these inhibitory effects are temporary. Aqueous and gaseous ammonia have been used to control microbial growth in stored fruits, hay, and grains. Ammonia treatment has proved more effective against fungal than against bacterial spoilage of food.

The bacterial species *Escherichia coli* and *Bacillus subtilis* were found to be sensitive to ammonium chloride (NH_4Cl) (Deal et al. 1975); exposure to 1100 mg NH_3/litre killed 90% of an *E. coli* population in 78 min. *B. subtilis,* an aerobic, spore-forming bacterium, was destroyed in less than 2 h in a solution of 620 mg

NH_3/litre. Anthonisen et al. (1976) and Neufeld et al. (1980) studied NH_3 inhibition of the bacterium *Nitrosomonas* (which converts ammonium to nitrite) and the bacterium *Nitrobacter* (which converts nitrite to nitrate). The nitrification process was inhibited by NH_3 at a concentration of 10 mg/litre (Neufeld et al., 1980). Concentrations that inhibited *Nitrosomonas* (10 - 150 mg/litre) were greater than those that inhibited *Nitrobacter* (0.1 - 1.0 mg/litre), and NH_3, not NH_4+, was reported to be the inhibiting chemical species (Anthonisen et al. 1976). Acclimatization of the nitrifying bacteria to NH_3, temperature, and the number of active nitrifying organisms are factors that may affect the inhibitory concentrations of NH_3 in a nitrification system.

Langowska & Moskal (1974) investigated the inhibitory effects of NH_3 on bacteria during 24-h exposure periods. Ammonifying and denitrifying bacteria were most resistant to NH_3; proteolytic and nitrifying bacteria were the most sensitive. Concentrations of up to 170 mg NH_3/litre did not adversely affect denitrifying and ammonifying bacteria; a concentration of 220 mg/litre caused a reduction in metabolic processes. Proteolytic bacteria were unaffected at concentrations of 0.8 mg NH_3/litre, but were affected at 13 - 25 mg/litre.

Jones & Hood (1980) conducted studies on 2 species of *Nitrosomonas* isolated from 2 wetland environments, one estuarine and the other fresh water. At 30 °C and pH 8.0, the estuarine isolate showed peak ammonium oxidation activity at 18 mg NH_3/litre; activity gradually declined to 30% of the peak at 80 mg NH_3/litre. However, the fresh-water isolate was not inhibited by ammonia concentrations of up to 80 mg NH_3/litre.

Application of anhydrous ammonia to soil may strongly affect soil microorganisms; however, the effect has been attributed more to alterations in pH than to ammonia toxicity *per se.* Henis & Chet (1967) found that ammonia reduced sclerotial germinability of the fungus *Sclerotium rolfsii* only when the soil pH rose to 9.8 or higher. According to Müller & Gruhn (1969), fungi were more sensitive than bacteria to ammonia application, the fungi disappearing at pH values above 8. At pH 8.38, bacterial numbers initially decreased but then increased above control levels, 7 days after application, with an increased number of protein-decomposing and nitrifying forms.

Ammonia has been used to control microbial growth in food and cattle feed. The growth of mould *(Penicillium digitatum)* on fresh fruit was inhibited by 70 - 300 mg ammonia/m^3 in the air, either applied directly or in the form of ammonia-releasing compounds (Eckert et al., 1963; Eckert, 1967). Anhydrous ammonia has also been used to prevent spoilage in high-moisture hay (Lechtenberg et al., 1977; Wilkinson et al., 1978; Butterworth, 1979) and cattle feed derived from corn leaves and stalks (Johanning et al., 1978). Ammonium hydroxide and ammonium isobutyrate were shown to control the growth of mould in stored corn (Bothast et al., 1973, 1975a,b; Rempel, 1975; Peplinski et al., 1978). Periodic addition of ammonia to stored corn, to a total of 30.4 g/kg, prevented the growth of mould, but was not fully effective as a bactericide (Peplinski et al., 1978).

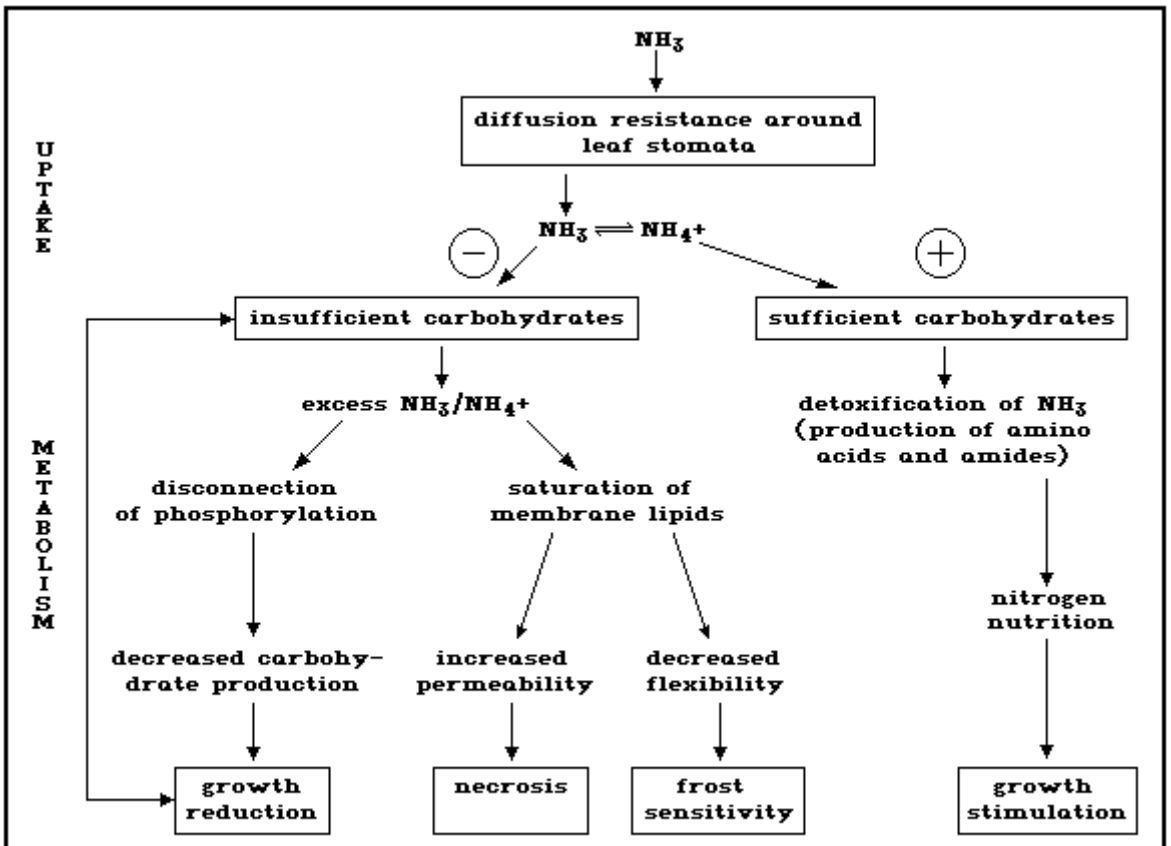
6.2. Plants

Ammonia may affect vegetation directly by acting on plant structure and function, and, indirectly, via its influence on soil

condition after being deposited. The effects of NH_3 are shown in Fig. 1.

6.2.1. Terrestrial plants

It is well recognized that nitrogen plays an important role in both plant metabolism and growth. The principal nitrogen sources are ammonium and nitrate ions. Ammonia is used primarily by the root system, but uptake of non-ionized ammonia or ammonium salts by the leaves also occurs. Ammonia is a nitrogen source for the synthesis of proteins. This use of ammonia in the synthesis of organic molecules can be regarded as a process for storing a valuable nutrient, but is also an important detoxifying mechanism. As ammonia is toxic, its uptake in large quantities can place a severe strain on the carbohydrate metabolism. Since carbohydrates are used in the synthesis of amino acids and amides, carbohydrate availability is an important factor in ammonia metabolism.



Effects of NH_3 on plants. From: Van der Erden (1982).

Manifestations of ammonia toxicity can be traced to several metabolic disturbances. Both photosynthetic and respiratory pathways are affected adversely by ammonia. There is a direct relationship between ammonia concentration and respiratory metabolism, including oxygen uptake, glycolysis, and the tricarboxylic acid cycle (Matsumoto et al., 1976). Ammonium ions may restrict photosynthesis through the uncoupling of noncyclic photophosphorylation in isolated chloroplasts (Gibbs & Calo, 1959; Losada & Arnon, 1963), though the mechanism of action is not known (Losada et al., 1973). It is well documented that uncoupling leads to an increase in reducing power in the cell. There is evidence that 1 - 3 mmol ammonium inhibits respiration in plants (Wedding & Vines, 1959; Vines & Wedding, 1960). It is suspected that similar or identical inhibition occurs in ammonium toxicity in animals. The site of toxicity is thought to be in the electron-transport

system; specifically, at the oxidation of NADH to NAD. This may have a mechanism similar to the interference by ammonium ion with electron transfer in the photosynthetic reaction.

High atmospheric levels may have direct toxic effects. Plant growth is inhibited by a decrease in the carbohydrate production as a consequence of inhibition of photosynthesis (Losada & Arnon, 1963). Ammonium increases the permeability of the cell membrane causing plasmolysis and necrosis. Furthermore, the flexibility of cells may be decreased, which results in an increased sensitivity to frost. Excess ammonia can be detoxified as long as amino acids are converted in the presence of carbohydrates (Fig. 1) (Van der Eerden, 1982).

Most data on the toxic exposure of plants to ammonia have been derived from controlled fumigation studies. Such experiments have provided the following relative susceptibilities to ammonia: leaves > stems, fungi, and bacteria < seeds, sclerotia, and animals (McCallan & Setterstrom, 1940).

An exposure of 175 mg/m^3 (250 ppm) for 4 min produced 50% foliar necrosis in tomatoes, whereas the same foliar injury was only produced in buckwheat and tobacco with exposure to 700 mg/m^3 (1000 ppm) for 5 and 8 min, respectively (Thornton & Setterstrom, 1940). Zimmerman (1949) reported that fumigation with ammonia at 28 mg/m^3 (40 ppm) for 60 min injured tomato, sunflower, and coleus completely, and a concentration of 2.1 mg/m^3 (3 ppm) severely injured mustard (Benedict & Breen, 1955). Other plant parts are more resistant to ammonia injury than the foliage. Thornton & Setterstrom (1940) found 50% injury in tomato stems after exposure to 700 mg/m^3 (1000 ppm) for 60 min. Barton (1940) observed that moist spring rye seeds were killed in a 4-h exposure to 700 mg ammonia/m^3 (100 ppm), whereas moist radish seeds were still viable after 16 h. Exposure to a concentration of 175 mg/m^3 (250 ppm) for 16 h reduced germination of rye seeds by half, but had no effect on radish seeds.

Foliar injury is the most common toxic effect of anhydrous ammonia on vegetation (Linzon, 1971). In broad-leaved woody plants exposed to high concentrations of ammonia, the injury begins as

large, dark green water-soaked areas on leaves, which darken into black or brownish-gray bifacial necrotic lesions, widely distributed over the leaf surface. In lightly injured leaves, these symptoms occur mainly on the upper surface. Conifer foliage injured by ammonia exposure darkens to shades of gray-brown, purple, or black. The entire needle is usually affected. Abscission of severely damaged leaves is often seen in broad-leaved and conifer species. On trees or shrubs with crowded leaves, injury may be confined to particular sections of the leaf. Occasionally, the foliage of woody species turns a variety of colours, mimicking autumn colours. Symptoms of injury in herbaceous plants are more variable, ranging from irregular, bleached, bifacial, necrotic lesions to dark upper-surface discolouration (Treshow, 1970). Grasses and cereal grains develop tan to reddish-brown marginal or interveinal necrosis, and broad-leaved weeds show red-brown to dark-brown upper surface discolouration on terminal or marginal portions of the leaf (Benedict & Breen, 1955). Coleus leaves lose their brilliant colour after exposure to ammonia and appear green (Zimmerman, 1949). Injury to flowers by ammonia is rarely seen in the field, but the development of small necrotic spots on azalea flowers has been reported following ammonia exposure (Treshow, 1970).

Although parts of plants, other than the foliage, are less susceptible to the injurious effects of ammonia (McCallan & Setterstrom, 1940), injury to apples, peaches, and other fruits and vegetables, accidentally exposed during cold storage, has been reported (Ramsey, 1953). Apparently, ammonia entered the fruit and turned red-pigmented tissues black, or brown, and yellow tissues, dark-brown. Immediately on exposure to ammonia, the outer skins of red onions became greenish-black, and the skin of yellow and brown onions became dark brown. These colour changes were usually permanent. Fumigation of fruit with ammonia also caused overall darkening of the skin. Peaches and apples showed such symptoms at 140 mg/m^3 (200 ppm) and 210 mg/m^3 (300 ppm), respectively (Brennan et al., 1962). These symptoms of injury were similar to those seen on fruits injured by the accidental release of ammonia.

In studies in the Netherlands, several vegetables and trees showed leaf damage by necrosis, growth reduction, and increased frost senstivity at concentrations of 75 µg/m^3 (annual average), 600 µg/m^3 (during 24 h), and 10 000 µg/m^3 (during 1 h) (Van der Eerden, 1982). The sensitivity of pine trees to exposure to ammonia differed between species (den Boer & Bastiaens, 1984). A survey is given in Table 14. Airborne ammonium sulfate deposits damaged the needles of pine trees. Owing to the enhanced uptake of ammonium, the excretion of potassium, magnesium, and calcium increases, often resulting in potassium and/or magnesium deficiency, which may lead to premature shedding of needles (Roelofs et al., 1985).

Effects of ammonia on the root system have generally been studied by exposing the roots to different aqueous ammonium solutions.

Table 14. Relative sensitivity of some pine trees to NH_3[a]

High sensitivity	Moderate sensitivity	Low sensitivity
Picea abies	*Picea omorika*	*Pinus sylvestris*
Picea sitchensis	*Pinus* nigra var.	*Pinus nigra nigra*
Taxus	maritima	*Tsuga canadensis*
Cupressus leylandii	*Taxus baccata*	*Taxus media*
	Pseutsuga menziesii	*Pinus mugo* var. mughus

[a] From: den Boer & Bastiaens (1984).

Exposure of tomato seedlings to ammonium solutions as the sole nitrogen source showed reduced growth. The root system was sparsely branched and discoloured and the stem was easily bruised. There was considerable wilting of leaves, which developed marginal necrosis (Pierpont & Minotti, 1977). Earlier, Maynard & Barker (1969), using cucumber *(C. sativus),* bean *(P. vulgaris),* and pea *(Pisum sativum* L.) in sand culture, demonstrated that ammonium toxicity was generally characterized by an immediate reduction in growth rate, wilting, marginal necrosis, interveinal chlorosis of terminal leaves and, finally, death of the entire plant. However, these symptoms did not occur in the ammonium medium with added calcium carbonate ($CaCO_3$) (Maynard & Barker, 1969; Pierpont & Minotti, 1977). Several studies have shown that an increase in the ammonium concentration is more deleterious to root than to shoot growth (Bennett et al., 1964; Haynes & Goh, 1977). Warncke & Barber (1973) observed that the ratio of root dry weight to shoot dry weight decreased significantly with increasing concentrations of nitrogen, but was not affected by the ammonium-to-nitrate ratio.

The roots appeared darker and less branched. The authors attributed the observed decrease in root production to greater acidity around the roots of ammonium-fed plants (Klemm, 1967; Warncke & Barber, 1973).

In the Netherlands, field observations and experiments have shown effects of ammonia and ammonium on pine forests, and heathland vegetation (Heil & Diemont, 1983; Van Breemen & Jordens, 1983; den Boer & Bastiaens, 1984; Roelofs, in press; Schuurkes, in press). In the United Kingdom, ombrotrophic mires (upland bogs deriving water only from rain) are affected by eutrophication (Lee, 1985). Forest ecosystems in the Federal Republic of Germany may also suffer from ammonia exposure (Hunger, 1978; Ulrich, 1983). Although only little information is available for other countries, it is expected that similar effects may occur in Belgium, Denmark, and the northwestern part of France, where high ammonia emissions occur as a consequence of the production of animal manure (Buysman et al., 1985).

The condition of forests may deteriorate as a consequence of direct exposure to ammonia. Pine trees in areas near intensive livestock farms are particularly affected (Hunger, 1978; Janssen, 1982). In the Netherlands, there is a clear correlation between ammonia emission and forest condition (Van Aalst, 1984). The damage observed is the result of both direct and indirect effects, and it is often difficult to distinguish between the two.

Field observations and experiments have shown deleterious effects on pine trees, namely:

- decreased vitality (growth reduction and necrosis);
- higher susceptibility to fungal diseases and attack by insects; and
- increased sensitivity to meteorological stress factors, e.g., hard frost.

Most of these phenomena can be explained in terms of disturbed nutrient budgets in trees. Enhanced ammonium sulfate uptake results in the excretion of essential nutrients, such as potassium and magnesium, by the needles, and the uptake of these ions by the root system is inhibited as a result of ammonium accumulation and cation leaching in soils (den Boer & Bastiaens, 1984; Roelofs, in press). Increased ammonium uptake ultimately leads to enhanced nitrogen levels in the leaves of beech (Nihlgard, 1970) and pine tress (Roelofs et al., 1985).

More attention is being paid to the role of ammonium in the forest dieback in Europe (Nihlgard, 1985).

Heather communities on poorly-buffered, slightly-acidic, and nutrient-poor heathland soils are also disturbed as a result of the deposition of airborne ammonia compounds, the number of plant species declining in acidified and nitrogen-enriched heathland soils. A succession from heather-dominated to grass-dominated heathlands has been observed (Heil & Diemont, 1983; Roelofs, in press). The same effects can be expected in other western European countries where similar plant communities are present.

6.2.2. Aquatic plants

In the aquatic environment, nitrogen plays an important role in determining the composition of phytoplankton and vascular plant

communities; in some cases, it can act as a limiting factor in primary production. Ammonia is important in nitrogen metabolism, because it functions as a nitrogen source in the synthesis of amino acids.

Most species can use either ammonium or nitrate as the sole nitrogen source, though, when both forms are available, ammonium is used first. Uptake takes place through both roots and leaves. The relative importance of ammonium as a nutrient depends on the absolute concentration and the ratio of ammonium to nitrate. Assimilation of ammonium is less expensive in terms of energy than nitrate, because the first metabolic step in which nitrate is reduced is not needed. Although ammonia is an important nutrient, it appears to be toxic at higher concentrations. Its uptake in large quantities may put a severe strain on the carbohydrate metabolism of the species, because carbon skeletons are used for detoxification (Bidwell, 1974). Increasing ammonia levels within the cell inhibit the utilization of nitrate. Ammonia solutions seem to be more toxic at high than at low pH, indicating that toxicity is probably due primarily to NH_3 rather than to NH_4+.

Surface waters that are poorly buffered, nutrient poor, and hydrologically dependent on rainfall and/or snow melt are most sensitive to ammonia. Deposition of ammonia and other nitrogen compounds may contribute significantly to the nitrogen enrichment of susceptible waters. Ombrotrophic mire plant communities can be altered by this atmospheric pollutant (Lee, 1985). In these *Sphagnum*-dominated wetlands, seed plants become more dominant. In small, poorly-buffered and nutrient-poor clear water lakes, both water composition and macrophyte composition are altered. Atmospheric ammonia deposition plays a major role in the acidification and nitrogen enrichment of surface waters in the Netherlands (Schuurkes, in press). Typical plant species belonging to the Littorellion alliance disappear from acidified waters. These species may be supressed by the luxuriant growth of *Sphagnum* species and *Juncus bulbosus.* Ammonium enrichment enables the last two species to form extremely high biomasses (Roelofs et al., 1984; Schuurkes, in press; Schuurkes et al., 1985). It should be noted that these changes in the plant community also influence the structure of the animal population.

6.2.3. Fresh-water plants

Experimental data concerning the toxicity of ammonia for fresh-water phytoplankton are limited. Przytocka-Jusiak (1976) reported the effects of ammonia on the growth of *Chlorella vulgaris.* A 50% inhibition was seen in 5 days of exposure to a concentration of 2.4 mg NH_3/litre; complete growth inhibition occurred in 5 days at 5.5 mg/litre. The NH_3 concentration resulting in 50% survival of *C. vulgaris* after 5 days was found to be 9.8 mg/litre. A *C. vulgaris* strain in which the tolerance to elevated ammonia concentrations was enhanced by prolonged incubation of the alga in ammonium carbonate solutions was isolated by Przytocka-Jusiak et al. (1977). *C. vulgaris* was reported to grow well in solutions containing 4.4 mg NH_3/litre, but growth was inhibited at 7.4 mg/litre (Matusiak, 1976). Tolerance to elevated concentrations of NH_3 seemed to show a slight increase, when other forms of nitrogen were available to the alga, rather than when ammonia was the only form of nitrogen in the medium. Bretthauer (1978) found that a concentration (assuming pH 6.5 and 30 °C) of 0.6 mg NH_3/litre killed *Ochromonas sociabilis,* and that, at 0.3 mg/litre, development of the population was reduced. Concentrations of 0.06 to 0.15 mg NH_3/litre had an insignificant effect on growth, and

concentrations of 0.015 to 0.03 mg/litre enhanced growth.

Ammonia at concentrations exceeding 2.5 mg NH_3/litre inhibited photosynthesis and growth in the algal species *Scenedesmus obliquus* and inhibited photosynthesis in the algae *Chlorella pyrenoidosa, Anacystis nidulans,* and *Plectonema boryanum* (Abelovich & Azov, 1976). Mosier (1978) reported that NH_3 concentrations causing a 50% reduction in oxygen production by the green alga *Chlorella ellipsoidea* and blue-green alga *Anabaena subcylindrica* were 16.0×10^{-8} and 251.0×10^{-8} µg NH_3-N/cell, respectively.

The rate of photosynthesis in the blue-green alga *P. boryanum* was stimulated by NH_4+, but inhibited by NH_3 (Solomonson 1969); the magnitude of these effects was dependent on the sodium-potassium composition of the suspension media. Inhibition of photosynthesis by NH_3 was associated with a conversion of inorganic polyphosphate, stored in the cells, to orthophosphate.

Champ et al. (1973) treated a small natural water pond with ammonia to achieve a mean concentration of 25.6 mg NH_3/litre. A diverse population of dinoflagellates, diatoms, desmids, and blue-green algae was present before ammonia treatment. Twenty-four hours after treatment, the mean number of phytoplankton cells/litre was reduced by 84%. By the end of 2 weeks (3.6 mg NH_3/litre), the original concentration of cells had been reduced by 95%.

Some research has been carried out to investigate the possible use of ammonia as an aquatic herbicide. Champ et al. (1973) reported virtually complete eradication of rooted aquatic vegetation (water shield, *Brasenia schreberi,* and American lotus, *Nelumbo* sp.). The NH_3 concentration was 25.6 mg/litre 24 h after ammonia addition, and 3.6 mg/litre, 2 weeks later. The use of high concentrations of ammonia to eradicate aquatic vegetation has been described by Ramachandran & Ramaprabhu (1976) and Ramachandran et al. (1975).

In experiments with *Potamogeton lucens,* Litav & Lehrer (1978) observed that ammonia caused appreciable injury to detached branches. Ammonia inhibition of the growth of Eurasian watermilfoil *(Myriophyllum spicatum)* affected both the length and weight of roots and shoots (Stanley, 1974).

Grube (1973) found that *Sium erectum* was slightly injured at 15 mg NH_3-N/litre, and completely eradicated at 35 mg/litre after a 10-week exposure. *Callitriche* sp. showed slight injury at 5 mg NH_3-N/litre, and the lethal dose was between 10 and 15 mg/litre. Injury was estimated from the amount of black colouring and the death of leaves. Roelofs et al. (1984) reported that exposure of the isoebid *Littorella uniflora* to 50 µmol NH_4+/litre for 10 weeks resulted in excretion of an equivalent amount of potassium, which may lead to discolouration and starvation.

Changes in the vegetation in 2 rivers subject to increased pollution from agricultural fertilizers, urban sewage, and industrial wastes, were studied by Litav & Agami (1976), who attributed the changes in the composition of the plant species primarily to a combination of ammonia and detergents. Agami et al. (1976) transplanted 7 species of "clean water" macrophytes to various sections of a river, and found that ammonia affected only *Nymphaea caerulea.*

6.2.4. Salt-water plants

A concentration of 0.24 mg NH_3/litre retarded the growth of

most of 10 species of benthic diatoms cultured for 10 days by Admiraal (1977). Pinter & Provasoli (1963) found that *Coccolithus huxleyi* was the most sensitive, and *Pavlova gyrans* and *Hymenomonas* sp. the most tolerant to ammonium sulfate with intermediate tolerance exhibited by *Syracosphaera* sp. and *Ochrosphaera neapolitana.*

Shilo & Shilo (1953, 1955) reported that the euryhaline alga *Prymnesium parvum* was effectively controlled by applications of ammonium sulfate, which exerted a lytic effect that decreased with increasing pH, indicating that NH_3 and not NH_4+ is responsible for the lytic activity of ammonium sulfate on *P. parvum.* It was reported by Byerrum & Benson (1975) that added ammonium ion at concentrations found to stimulate the photosynthetic rate also caused the alga *Amphidinium carterae* to release up to 60% of fixed $^{14}CO_2$ to the medium.

Natarajan (1970) found that the concentrations of fertilizer plant effluent that were toxic for natural phytoplankton (predominantly diatoms) ranged between 1.1 and 11 mg NH_3/litre. Thomas et al. (1980) concluded that increased ammonium concentrations found near sewage outlets would not be inhibiting to phytoplankton in the vicinity. Provasoli & McLaughlin (1963) reported that ammonium sulfate was toxic for some marine dinoflagellates, but only at concentrations far exceeding those in sea water.

6.3. Aquatic Invertebrates

The toxicity of ammonia has been less extensively studied in invertebrates than in fish. Most of the available invertebrate data consists of studies on arthropods, primarily crustaceans and insects. Many of these studies are laboratory tests in which test animals were exposed to known concentrations of a toxic agent for specified periods of time. Results may be expressed as a median lethal concentration (LC_{50}) for a given time period (e.g., 48-h LC_{50}) or, occasionally, as an effective concentration (EC), that is, the concentration at which the test animal is completely immobilized, though it might still be respiring (e.g., 48-h EC). Another method of reporting test results is as the estimated time required to kill 50% of a test population (LT_{50}) at a given concentration of toxin (e.g., LT_{50} = 250 min).

6.3.1. Fresh-water invertebrates: acute toxicity

The acute toxicity of ammonia for *Daphnia magna* has been studied by Parkhurst et al. (1979, 1981) and Reinbold & Pescitelli (1982a) with reported 48-h LC_{50}s values of 2.08 and 4.94 mg NH_3/litre. DeGraeve et al. (1980) reported a similar 48-h LC_{50} value for *Daphnia pulicaria* of 1.16 mg NH_3/litre. A threshold toxicity value for *D. magna* of 2.4 - 3.6 mg NH_3/litre lake water was reported by Anderson (1948). Threshold concentration was taken as the highest concentration that would just fail to immobilize the test animals, under conditions of prolonged exposure (Anderson, 1948). A minimum lethal concentration of 0.55 mg NH_3/litre was reported for *D. magna* by Malacea (1966), and a 24-h LC_{50} value of 1.50 mg NH_3/litre was reported by Györe & Oláh (1980) for *Moina rectirostris.*

Buikema et al. (1974) reported an EC_{50} for NH_3 toxicity in the rotifer, *Philodina acuticornis,* to be 2.9 - 9.1 mg NH_3/litre (calculated using reported pH values of 7.4 - 7.9). Tests of ammonia toxicity for the flatworm, *Dendrocoelum lacteum (Procotyla*

fluviatilis), and a tubificid worm *(Tubifex tubifex)* gave LC_{50} values of 1.4 and 2.7 mg NH_3/litre, respectively (Stammer, 1953).

Thurston et al. (1984a) conducted 25 flow-through toxicity tests with 3 mayfly, 2 stonefly, 1 caddisfly, and 1 isopod species; all tests were conducted with water of similar chemical composition. The 96-h LC_{50} values ranged from 1.8 to 5.9 mg NH_3/litre. The results also indicated that a 96-h test is not long enough to determine the acutely lethal effects of ammonia on the species tested, because an asymptotic LC_{50} was not always obtained within 96 h. Percentage survival data were reported for some mayfly, stonefly, and caddisfly tests in which LC_{50} values were not obtained; there was 60 - 100% survival at test concentrations ranging from 1.5 to 7.5 mg NH_3/litre. Gall (1980) tested NH_4Cl with *Ephemerella* sp. (near *excrucians*). Organisms were exposed to ammonia for 24 h, followed by 72 h in ammonia-free water; mortality observations were made at the end of the overall 96-h period. An EC_{50} value of 4.7 mg NH_3/litre was obtained. Hazel et al. (1979) reported a LC_{50} value of 8.0 mg NH_3/litre for the beetle *Stenelmis sexlineata.*

No deaths occurred in ammonia toxicity tests conducted on scud, *Gammarus lacustris,* or *D. magna,* using dilution water from a river, after a 96-h exposure to 0.08 mg NH_3/litre. In a second test, using river water buffered with sodium bicarbonate, 13% mortality occurred with scud at the several concentrations tested, including the highest and lowest of 0.77 and 0.12 mg NH_3/litre, respectively; 7 and 13% mortality occurred with *D. magna* at the same concentrations (Miller et al., 1981).

Five fresh-water mussel species, *Amblema p. plicata, Anodonta imbecillis, Corbicula manilensis, Cyrtonaias tampicoensis,* and *Toxolasma texasensis,* were exposed for 165 h to a concentration of 0.32 mg NH_3/litre; *T. texasensis* was most tolerant to ammonia, and *A. p. plicata* was most sensitive (Horne & McIntosh, 1979). During the tests, the more tolerant species generally had their shells tightly shut, whereas the least tolerant species continued siphoning or had their mantles exposed. In 2 studies, acute exposure of the fresh-water crayfish, *Orconectes nais,* to ammonium chloride gave LC_{50} values of 3.15 and 3.82 mg NH_3/litre, respectively (Evans, 1979; Hazel et al., 1979).

6.3.2. Fresh-water invertebrates: chronic toxicity

Few studies have been conducted on the long-term exposure of fresh-water invertebrates to ammonia. In a long-term test conducted by Reinbold & Pescitelli (1982a), reproduction and growth of *D. magna* were affected at a concentration of 1.6 mg NH_3/litre.

Two tests lasting 42 days were conducted by Anderson et al. (1978) on the effects of ammonium chloride on the fingernail clam, *Musculium transversum.* Significant mortality (67 and 72%) occurred in both tests at a concentration of 0.7 mg NH_3/litre. In one of the studies, significant reduction in growth was observed after 14 days of exposure to 0.41 mg NH_3/litre. Sparks & Sandusky (1981) reported that fingernail clams exposed to 0.23 and 0.63 mg NH_3/litre showed 36 and 23% mortality, respectively, in 4 weeks; after 6 weeks, there was 47% mortality at 0.073 mg NH_3/litre, and 83% mortality at 0.23 and 0.63 mg NH_3/litre. After 6 weeks there was no growth in any test chamber (concentrations of 0.036 mg NH_3/litre and higher), other than in the controls.

Two partial tests, lasting 24 and 30 days, respectively, were

conducted by Thurston et al. (1984a) on the stonefly *Pteronarcella badia.* Adult stonefly emergence was delayed with increasing ammonia concentration, and little or no emergence occurred at concentrations exceeding 3.4 mg NH_3/litre. There was no significant relationship between the food consumption rates of nymphs and concentrations up to 6.9 mg NH_3/litre. LC_{50} values for 24- and 30-day exposures were 1.45 and 4.57 mg NH_3/litre, respectively.

The effects of ammonia on the ciliary beating rate of clam gills were investigated by Anderson et al. (1978). Concentrations of 0.036 - 0.11 mg NH_3/litre caused a reduction in the ciliary beating rate of fingernail clams; the effects ranged from a 50% reduction in beating rate to complete inhibition. Adult clams (> 5 mm) were more sensitive than juveniles ($\leq$ -5 mm); adults were also slightly more sensitive than the unionid mussel, *Elliptio complanata,* and the Asiatic clam, *C. manilensis.* Shaw (1960) investigated the effects of ammonium chloride on sodium influx in the fresh-water crayfish, *Astacus pallipes.* Ammonia produced an inhibition of sodium influx; a concentration of 18 mg NH_4+/litre reduced the influx to about 20% of its normal value, and influx reduction was related to increasing ammonia concentration. This effect was attributed to NH_4+ ions and not to any toxic effect exerted on the transporting cells by non-ionized ammonia. NH_4+ did not affect chloride influx nor the rate of sodium loss.

Ammonia was added to a stream at a 24-h average concentration of 1.4 mg NH_3/litre, and a 24-h drift net sampling was conducted (Liechti & Huggins, 1980). No change in diel drift pattern was observed, but there was an increase in the magnitude of drift, a shift in the kinds of organisms present, and changes in benthic standing crop estimates; this ammonia concentration was non-lethal.

6.3.3. Salt-water invertebrates: acute and chronic toxicity

Data on the acute toxicity of ammonia for salt-water invertebrate species are very limited. A 96-h LC_{50} value of 1.5 mg NH_3/litre has been reported for the copepod, *Nitocra spinipes* (Linden et al., 1979). Lethal effects of ammonium chloride on the quahog clam, *Mercenaria mercenaria,* and eastern oyster, *Crassostrea virginica,* were studied by Epifano & Srna (1975). There were no observed differences in susceptibility between

juveniles and adults of the 2 species. Armstrong et al. (1978) conducted acute toxicity tests (6 days) on ammonium chloride using prawn larvae, *Macrobrachium rosenbergii.* LC_{50} values were highly pH-dependent. The acute toxicity of ammonium chloride for penaeid shrimp was reported as a 48-h composite LC_{50} value of 1.6 mg NH_3/litre for 7 species pooled, including the resident species *Penaeus setiferus* (Wickins, 1976). The acute toxicity of ammonium chloride for the caridean prawn, *M. rosenbergii,* was reported (Wickins, 1976) as LT_{50} values of 1700 - 560 min at concentrations of 1.74 - 3.41 mg NH_3/litre. From the data of Hall et al. (1978), 48-h LC_{50} values of 0.34 - 0.53 were estimated for grass shrimp, *Palaemonetes pugio.* Catedral et al. (1977a,b) investigated the effects of ammonium chloride on the survival and growth of *Penaeus monodon;* larvae had a lower tolerance to ammonia compared with post-larvae. Brown (1974) reported a time to 50% mortality of 106 min for the nemertine worm, *Cerebratulus fuscus,* at a concentration of 2.3 mg NH_3/litre.

Effects of ammonium chloride solutions on the American lobster, *Homarus americanus,* were studied by Delistraty et al. (1977). Their studies were performed on fourth-stage larvae, which was

considered to be the most sensitive life stage. A 96-h LC_{50} value of 2.2 mg NH_3/litre and an incipient LC_{50} value of 1.7 mg NH_3/litre were reported. A "safe" concentration of 0.17 mg NH_3/litre was tentatively recommended.

The sublethal toxicity of ammonium chloride for the quahog clam and eastern oyster was studied by Epifano & Srna (1975) who measured the effect of a 20-h exposure to ammonia on the rate of removal of the alga, *Isochrysis galbana,* from suspension (clearing rate) by the clams and oysters. Concentrations of 0.06 - 0.2 mg NH_3/litre affected removal; no differences were observed between juveniles and adults. The effect of ammonia on the ciliary beating rate of the mussel, *Mytilus edulis,* was studied by Anderson et al. (1978). Concentrations of 0.097 - 0.12 mg NH_3/litre resulted in a reduction in the ciliary beating rate ranging from 50% to complete inhibition.

Exposure of unfertilized sea urchin, *Lytechinus pictus*, eggs to ammonium chloride resulted in stimulation of the initial rate of protein synthesis, an event that normally follows fertilization (Winkler & Grainger, 1978). Exposure of unfertilized eggs of *Strongylocentrotus purpuratus, L. pictus, and Strongylocentrotus drobachiensis* to ammonium chloride (Johnson et al., 1976; Paul et al., 1976) caused "fertilization acid" to be released more rapidly and in greater amounts than after insemination. Activation of unfertilized *L. pictus* eggs by ammonium chloride exposure was also evidenced by an increase in intracellular pH (Steinhardt & Mazia, 1973; Shen & Steinhardt, 1978). Ammonia treatment has also been reported to activate phosphorylation of thymidine and synthesis of histones in unfertilized eggs of the sea urchin, *S. purpuratus,* (Nishioka, 1976). Premature chromosome condensation was induced by ammonia treatment of eggs of *L. pictus* and *S. purpuratus* (Epel et al., 1974; Wilt & Mazia, 1974; Krystal & Poccia, 1979). Treatment of *S. purpuratus and S. drobachiensis* fertilized eggs with ammonia resulted in an absence of the normal uptake of calcium following insemination. However, calcium uptake was not inhibited when ammonia treatment preceded insemination (Paul & Johnston, 1978).

The polychetous annelid, *Nereis succinea,* the channelled whelk, *Busycon canaliculatum,* and the brackish-water clam, *Rangia cuneata,* were exposed to concentrations of 0.85, 0.37, and 0.27 mg NH_3/litre and the ammonia excretion measured (Mangum et al., 1978). Excretion was inhibited by non-lethal concentrations of ammonia, and the authors concluded that ammonia crosses the excretory epithelium in the ionized form, and that the process is linked to the activity of the Na^++K^+ ATPases. When blue crabs, *Callinectes sapidus,* were moved from water of 28 parts per thousand salinity to water of 5 parts per thousand, a doubling of the ammonia excretion rate occurred; addition of excess ammonium chloride to the low-salinity water inhibited ammonia excretion and decreased net acid output (Mangum et al., 1976). The effect of gaseous NH_3 on haemoglobin from the blood of the common marine bloodworm, *Glycera dibrachiata,* was examined by Sousa et al. (1977) in an attempt to determine whether there was competition between NH_3 and oxygen in binding to haemoglobin; such an NH_3/O_2 relationship was not found.

6.4. Fish

Ammonia is highly toxic for fish, and, because of its occurrence at high concentrations in some water systems, it can present a major pollution problem. It enters aquatic environments

from several sources, including sewage effluent, deposition of human wastes without treatment, industrial discharges, and runoff from animal culture and agricultural operations. It is also a metabolic waste product of fish and, therefore, can be a problem in facilities involved with intensive fish culture.

Elevated ammonium ion (NH_4+) concentrations within the bodies of fish, as with other vertebrates, cause convulsions and death. The concentration of non-ionized ammonia (NH_3) in the environment of the fish is important, because ammonia is transferred between the water and fish largely in this form. Thus, while NH_3 is the more toxic chemical species in the water, within the fish, toxicity is related to the NH_4 concentration.

Research by Chipman (1934), Wuhrman et al. (1947), Wuhrman & Woker (1948), and Tabata (1962) implicated NH_3 as the ammonia species in water that is mainly toxic for fish, and reported that NH_4+ was non-toxic or considerably less toxic. More recent research by Robinson-Wilson & Seim (1975), Armstrong et al. (1978), and Thurston et al. (1981c) has demonstrated that the role of water pH in the toxicity of ammonia for fish is more than the regulation of the NH_3/NH_4+ equilibrium. NH_3 is considerably more toxic in water when pH values are lower than 7 - 9, and there is some evidence that the toxicity of NH_3 is also increased above this range. Temperature and dissolved oxygen and the ionic composition of the background water all play a role in the toxicity of NH_3 for some fish.

In a comprehensive analysis of the data, it was concluded that a number of fish species that are phylogenetically similar are also similar in their sensitivity to the toxicity of ammonia, and that many of the factors that affect the toxicity of ammonia similarly affect all the species that have been studied (US EPA, 1985).

It was formerly considered that fish of the family Salmonidae were among the most sensitive to the effects of many pollutants, whereas other fish species, which have evolved in warm-water, low oxygen, or more turbid aquatic environments, may be less sensitive to many naturally-occuring pollutants, such as ammonia. However, other fish species, including some that are frequently referred to as "warm water" fishes, are of comparable sensitivity (US EPA, 1985). In the "resident-species" approach for establishing water quality criteria for a toxic agent in a given water body, the tolerance of selected fish and invertebrate species, naturally resident in the water body, is used. It must be borne in mind that, if these selected species are less sensitive than other species in different water bodies, the standards may be inappropriate for other water bodies.

Because of variation among different background test water conditions in different laboratories, as well as differences in the genetic pools of the same species, results of a single test on a given species may not be as meaningful as composite results from several tests conducted at different laboratories.

Much of the evidence on ammonia toxicity is empirical, so, for a more complete understanding of the biological actions of this chemical, the results of toxicity tests must be integrated with a knowledge of ammonia metabolism in fish.

6.4.1. Ammonia metabolism in fish

6.4.1.1. Ammonia production and utilization

The major pathway for the production of ammonia in fish, as in other vertebrates, is through the transamination of various amino acids (Forster & Goldstein, 1969; Watts & Watts, 1974). The primary site for ammonia production is probably the liver (Pequin & Serfaty, 1963), but the necessary enzymes have also been located in the kidneys, gills, and skeletal muscle tissue (Goldstein & Forster, 1961; McBean et al., 1966; Walton & Cowey, 1977). Ammonia is also produced by the deamination of adenylates in fish muscle (Driedzic & Hochachka, 1976). The quantitative importance of muscle ammoniogenesis in total ammonia excretion depends on the level of activity of the animal, and increases with increasing workload (Suyama et al., 1960; Fraser et al., 1966; Driedzic & Hochachka, 1976).

Ammonia toxicity can be ameliorated by the formation of less toxic compounds, namely glutamine and urea. Levi et al. (1974) recorded high levels of glutamine in the brain of goldfish, *Carrasius auratus,* and found that brain-glutamine levels increased with ambient ammonia concentrations. Webb & Brown (1976) found high glutamine synthetase (EC 6.3.1.2) activity in the brains of teleosts and elasmobranchs, and this may be important in protecting the brain from sudden surges in ammonia concentration. Walton & Cowey (1977) were able to detect glutaminase activity in the gills of rainbow trout, but were unable to measure any *in vivo* utilization of glutamine by the gills.

Ammonia can be converted, through carbamyl phosphate, to urea either via purines (uricolysis) or via the ornithine cycle. The enzymes required for uricolysis have been found in most fish studied (Forster & Goldstein, 1969; Watts & Watts, 1974), but Florkin & Duchateau (1943) were unable to detect any activity of uricolytic enzymes in the cyclostome, *Lampetra.* The ratio of urea production via the ornithine cycle to production via uricolysis is about 100 to 1 in elasmobranchs and dipnoi, whereas in teleosts most of the urea is formed via uricolysis (Gregory, 1977).

6.4.1.2. Ammonia excretion

The gills are the major site of ammonia excretion in fish, but smaller quantities of ammonia may also be eliminated by the kidneys (Edwards & Condorelli, 1928; Grollman, 1929; Fromm, 1963; Maetz, 1972) and skin (Morii et al., 1978). Although the majority of branchial ammonia excretion represents clearance from the blood, gill metabolism may contribute between 20% (Payan & Matty, 1975) and 5 - 8% (Cameron & Heisler, 1983) of the net ammonia excretion.

The excretion of ammonia by fish is variable, depending on the state of the animal, the environmental conditions, and the species. Ammonia excretion tripled in sockeye salmon, *Oncorhynchus nerka,* following daily feeding (Brett & Zala, 1975) but remained low and unchanging during starvation (Brett & Zala, 1975; Guerin-Ancey, 1976a). In fresh-water fish, ammonia excretion increases in response to exercise (Sukumaran & Kutty, 1977; Holeton et al., 1983), long-term acid exposure (McDonald & Wood, 1981; Ultsch et al., 1981), hypercapnia (Claiborne & Heisler, 1984), and NH_4Cl infusion (Hillaby & Randall, 1979). In contrast, increased levels of environmental ammonia (Guerin-Ancey, 1976b) and short-term exposure to acid or alkaline water (Wright & Wood, 1985) cause a decrease in ammonia excretion. It is not known if these changes in excretion reflect change in the rate of ammonia production or in the ammonia content of the body. The ammonia content of fish is likely to be the equivalent of the ammonia excreted in about 2 h, most of the ammonia being in the tissues with a lower pH, such as

muscle. Blood levels are around 0.2 to 0.3 mmol, but muscle at a lower pH may contain levels of up to 1 mmol; thus, a 1-kg fish may contain about 0.5 to 0.7 mmol of ammonia and have an excretion rate of about 0.3 mmol/h. There is increased ammonia production in muscle during exercise (Driedzic & Hochachka, 1976). Ammonia excretion by the spiny dogfish, *Squalus acanthias,* in sea water is unaffected by temperature change, exercise, hyperoxia, hypercapnia, or the infusion of either HCl or $NaHCO_3$ or anything that induces acid-base stress (Heisler, 1984). This is surprising, because many of these changes affect pH and therefore would be expected to alter the ammonia content of body compartments and consequently ammonia excretion.

There is an elevation in blood-ammonia during starvation (Morii et al., 1978; Hillaby & Randall, 1979), even though ammonia excretion does not change (Brett & Zala, 1975). Blood-ammonia concentrations also rise with increases in both temperature (Fauconneau & Luquet, 1979) and ammonia concentrations in the water (Fromm & Gillette, 1968; Thurston et al., 1984b). Exposure of fish to either air (Gordon, 1970) or increased ammonia levels in water (Fromm, 1970; Guerin-Ancey, 1976b), raises blood-ammonia levels and reduces ammonia excretion; this is associated with a rise in urea production in many, but not all fish. Unlike the authors of the above studies, Buckley et al. (1979) did not find any change in blood-total ammonia when coho salmon, *Oncorhynchus kisutch,* were exposed to elevated ammonia levels in the environment. However, a significant rise in plasma-sodium, indicating some coupling between sodium uptake and ammonia excretion, was observed.

The study of ammonia movement is complicated by the fact that, with present analytical techniques, it is impossible to distinguish between the transfer of a molecule of NH_3 plus a H^+ ion from the transfer of an NH_4+ ion. Thus, only indirect evidence can be obtained regarding the relative gas and ion movements across the gill epithelium.

Three possible mechanisms of ammonia excretion have received the most attention: passive NH_3 flux, ionic exchange of NH_4+ for Na^+, and passive NH_4+ flux. There seems to be little doubt that a significant pathway for branchial ammonia excretion is by the passive diffusion of NH_3 down its partial pressure gradient. Changes in the NH_3 partial pressure gradient are positively correlated with changes in net ammonia excretion in the channel catfish, *Ictalurus punctatus*, (Kormanik & Cameron, 1981), and rainbow trout (Cameron & Heisler, 1983; Wright & Wood, 1985). Ammonia entry into the fish has also been shown to be dependent on the NH_3 gradient (Wuhrmann et al., 1947; Wuhrmann & Woker, 1948; Fromm & Gillette, 1968).

The excretion of NH_4+ is strongly coupled with the movement of other ions. Many studies have attempted to link the transepithelial exchange of Na^+ uptake to NH_4+ efflux. Although there is considerable indirect evidence for the presence of a coupled ionic exchange mechanism under certain conditions (Maetz & Garcéa-Romeu, 1964; Maetz, 1973; Payan & Maetz, 1973; Evans, 1977, 1980; Payan, 1978; Girard & Payan, 1980; Wright & Wood, 1985), the ubiquity and stoichiometry of this exchange remain controversial. While Na^+ influx can be monitored with isotopes, it is difficult to determine NH_4+ efflux. Investigators have attempted to quantify the relationship between Na^+ uptake and NH_4+ excretion by manipulating Na^+ levels in the environmental water, by pharmaceutical inhibition of the Na^+ influx mechanism, or by loading the fish with ammonia.

In goldfish (Maetz, 1973), and in irrigated rainbow trout gills (Kirschner et al., 1973), Na^+ influx was best correlated with the sum of H^+ and NH_4+ ion efflux. The possibility of a Na^+ uptake carrier coupled to either NH_4+ or H^+ appears likely in other fish as well (Kerstetter et al., 1970; Payan & Maetz, 1973; Evans, 1977). In perfused heads of trout, Na^+ uptake was tightly coupled with NH_4+ efflux (Payan et al., 1975; Payan, 1978). Wright & Wood (1985) demonstrated that, in intact trout, the rate of ion exchange was influenced by external water pH, increasing from no exchange at pH 4 to maximal rates at pH 8. The relationship between Na^+ and NH_4+ was one-to-one at a pH of less than 8. However, Payan et al. (1975) and Wright & Wood (1985) found that the majority of the ammonia was eliminated by gaseous diffusion, and only when NH_3 was subtracted from total ammonia efflux was the NH_4+/Na^+ exchange evident. In common carp, *Cyprinus carpio* (de Vooys, 1968), and little skate, *Raja erinacea* (Evans et al., 1979), ammonia excretion was unaffected by a reduction in environmental Na^+ levels. Cameron & Heisler (1983) found that, under resting conditions, diffusive movement of NH_3 could account for ammonia excretion in trout, but when the ammonia gradient was reversed and directed inwards, an NH_4+/Na^+ exchange could counter-balance the diffusive uptake of NH_3 from the water. If this hypothesis is correct, then it would explain the unchanging blood-ammonia levels and increased Na^+ levels in coho salmon exposed to elevated concentrations of ammonia in the water (Buckley et al., 1979).

The Na^+/NH_4+ (H^+) exchange is probably located on the epithelial apical membrane. Either acid conditions or amiloride in the water inhibits Na^+ influx across the gills and both these conditions result in a reduction in ammonia excretion (Wright & Wood, 1985).

The ammonium ion can displace potassium in many membrane processes in, for example, the giant axon of squid, *Loligo pealei* (Binstock & Lecar, 1969), and this is the probable reason that elevated ammonia causes convulsions in so many vertebrates. In various aquatic animals, it is possible that NH_4+ can substitute for potassium in oubain-sensitive sodium/potassium exchange (Payan et al., 1975; Towle & Taylor, 1976; Towle et al., 1976; Mallery, 1979; Girard & Payan, 1980). NH_4+ ions will substitute for K^+ ions across the epithelial basolateral border (Richards & Fromm, 1970; Shuttleworth & Freeman, 1974; Karnaky et al., 1976), but the importance to net ammonia transfer in fresh-water fish is unknown.

The passive movement of NH_4+ down its electrochemical gradient may also contribute to net ammonia excretion (Claiborne et al., 1982; Goldstein et al., 1982). Lipid membranes are relatively impermeable to cations (Jacobs, 1940) and, because respiratory epithelial cells of fresh-water fishes are joined by tight junctions (Girard & Payan, 1980), it appears unlikely that NH_4+ diffusion is of quantitative importance (Kormanik & Cameron, 1981). Indeed, Wright & Wood (1985) found a negative correlation between ammonia excretion and the NH_4+ concentration gradient in rainbow trout exposed to 5 different water pH regimes. Although it appears that NH_4+ diffusion may be of minor importance, simultaneous measurements of the electrical and chemical gradient have not been made and are necessary before conclusions can be drawn.

6.4.2. Fish: acute toxicity

The acute toxicity of ammonia for rainbow trout has been studied by many investigators, with reported 96-h LC_{50} values ranging from 0.16 to 1.1 mg NH_3/litre. Thurston & Russo (1983) conducted 71 toxicity tests on rainbow trout ranging in size from sac fry (< 0.1 g) to 4-year-old adults (2.6 kg), in water of uniform chemical composition. LC_{50} values ranged from 0.16 to 1.1 mg NH_3/litre for 96-h exposures. Fish susceptibility to NH_3 decreased with increasing weight over the range 0.06 - 2.0 g, but gradually increased above that weight range. LC_{50} values for 12- and 35-day exposures did not differ greatly from 96-h values. No statistically-significant differences in results were observed when different ammonium salts [NH_4Cl, NH_4HCO_3, $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$] were used. Grindley (1946) also reported that there were no appreciable differences in toxicity between toxic solutions of NH_4Cl and $(NH_4)_2SO_4$ in rainbow trout tests. However, Calamari et al. (1977, 1981) reported that embryos and fingerlings were less sensitive than the other life stages studied. LC_{50} values (96-h) ranging from 0.16 to 1.02 mg NH_3/litre for rainbow trout exposed to ammonia were reported by Calamari et al. (1977, 1981), Broderius & Smith (1979), Holt & Malcolm (1979), DeGraeve et al. (1980), and Reinbold & Pescitelli (1982b).

Although acute toxicity studies with salmonids have mainly been conducted on rainbow trout, some data are available for a few other salmonid species. Thurston et al. (1978) investigated the toxicity of ammonia for cutthroat trout, *Salmo clarki,* and reported 96-h LC_{50} values of 0.52 - 0.80 mg NH_3/litre. Thurston & Russo (1981) reported a 96-h LC_{50} value of 0.76 mg NH_3/litre for golden trout, *Salmo aguabonita*. Brown trout, *Salmo trutta,* were exposed to 0.15 mg NH_3/litre for 18 h, resulting in a 36% mortality; when returned to ammonia-free water, the test fish recovered after 24 h (Taylor, 1973). No mortality occurred during the 96-h exposure at 0.090 mg NH_3/litre, although fish would not feed. Exposure to 0.8 mg NH_3/litre was not acutely toxic for brown trout according to Woker & Wuhrmann (1950). However, Thurston & Meyn (1984) reported 96-h LC_{50} values of 0.60 - 0.70 mg NH_3/litre, and Miller et al. (1981) reported a 96-h LC_{50} value of 0.47 mg NH_3/litre for brown trout using test dilution river water. Brook trout, *Salvelinus fontinalis,* showed distress within 1.75 h at a concentration of 3.25 mg NH_3/litre and within 2.5 h at 5.5 mg/litre (Phillips, 1950). Thurston & Meyn (1984) reported 96-h LC_{50} values of 0.96 - 1.05 mg NH_3/litre for brook trout, 0.40 - 0.48 mg NH_3/litre for chinook salmon, *Oncorhynchus tshawytscha,* and 0.14 - 0.47 mg NH_3/litre for mountain whitefish, *Prosopium williamsoni.* Toxicity tests with $(NH_4)_2SO_4$ on pink salmon, *Oncorhynchus gorbuscha,* at different early stages of development (Rice & Baily, 1980) showed that late alevins near swim-up stage were the most sensitive (96-h LC_{50} = 0.083 mg NH_3/litre), and eyed embryos were the most tolerant, surviving 96 h at > 1.5 mg NH_3/litre. Buckley (1978) reported a 96-h LC_{50} value of 0.55 mg NH_3/litre for fingerling coho salmon, *Oncorhynchus kisutch,* and Herbert & Shurben (1965) reported a 24-h LC_{50} value of 0.28 mg NH_3/litre for Atlantic salmon, *Salmo salar.*

There are acute toxicity data for ammonia in a variety of non-salmonid fish species. Thurston et al. (1983) studied the toxicity of ammonia for fathead minnows, *Pimephales promelas*, ranging from 0.1 to 2.3 g in weight and found that 96-h LC_{50} values in 29 tests ranged from 0.75 to 3.4 mg NH_3/litre; toxicity was not dependent on the test fish size or source. LC_{50} values ranging from 0.73 to 2.35 mg NH_3/litre for fathead minnows were also reported by Sparks (1975), DeGraeve et al. (1980), Reinbold & Pescitelli (1982b), Swigert & Spacie (1983). LC_{50} values for white sucker, *Catostomus*

commersoni, exposed to ammonium chloride solutions for 96 h (Reinbold & Pescitelli 1982c) were 1.40 and 1.35 mg/litre NH_3, though a somewhat lower 96-h LC_{50} of 0.79 mg NH_3/litre was determined by Swigbert & Spacie (1983). Thurston & Meyn (1984) reported 96-h LC_{50} values of 0.67 - 0.82 mg NH_3/litre for the mountain sucker, *Caststomus platyrhynchus.*

Reported LC_{50} values for 96-h exposures of bluegill, *Lepomis macrochirus,* ranged from 0.26 to 4.60 mg NH_3/litre (Emery & Welch, 1969; Lubinski et al., 1974; Roseboom & Richey, 1977; Reinbold & Pescitelli, 1982b; Swigert & Spacie, 1983). LC_{50} values (96-h) of 0.7 - 1.8 mg NH_3/litre for smallmouth bass, *Micropterus dolomieui,* and 1.0 - 1.7 mg NH_3/litre for largemouth bass, *Micropterus salmoides,* were reported by Broderius et al. (in press) and Roseboom & Richey (1977), respectively. Sparks (1975) reported 48-h LC_{50} values for bluegill of 2.30 mg NH_3/litre, and for channel catfish of 2.92 mg NH_3/litre. For goldfish, *Carassius auratus,* Dowden & Bennett (1965) reported a 24-h LC_{50} of 7.2 mg NH_3/litre, and Chipman (1934) reported lethal threshold values of 0.97 - 3.8 mg NH_3/litre. Turnbull et al. (1954) reported a 48-h LC_{50} for bluegill to be within the range 0.024 - 0.093 mg NH_3/litre; during the exposure, they observed that the fish exhibited a lack of ability to avoid objects.

Reported 96-h LC_{50} values for channel catfish, *Ictalurus punctatus,* ranged from 1.5 to 4.2 mg NH_3/litre (Colt & Tchobanoglous, 1976; Roseboom & Richey, 1977; Reinbold & Pescitelli, 1982d; Swigert & Spacie, 1983). Vaughn & Simco (1977) reported a 48-h LC_{50} for channel catfish of 1.24 - 1.96 mg NH_3/litre, and Knepp & Arkin (1973) reported 1-week LC_{50} values of 0.97 - 2.0 mg NH_3/litre. The results of studies on bluegill, channel catfish, and largemouth bass (Roseboom & Richey, 1977) showed that bluegill susceptibility was dependent on fish weight, fish weighing 0.07 g being slightly more sensitive than those weighing either 0.22 or 0.65 g; size had little effect on the susceptibility of channel catfish or bass.

Hazel et al. (1979) reported 96-h LC_{50} values of 0.90 and 1.07 mg NH_3/litre for the orangethroat darter, *Etheostoma spectabile,* and red shiner, *Notropis lutrensis.* Largemouth bass, channel catfish, and bluegill were also exposed for 96 h to a concentration of 0.21 mg NH_3/litre resulting in zero mortality for bluegill and channel catfish and one death (6%) among the largemouth bass tested. A 96-h LC_{50} value for walleye, *Stizostedion vitreum,* of 0.85 mg/litre NH_3 was reported by Reinbold & Pescitelli (1982a).

LC_{50} values ranging from 2.4 to 3.2 mg NH_3/litre for $(NH_4)_2CO_3$, NH_4Cl, $NH_4C_2H_3O_2$, and NH_4OH, in 96-h exposures of mosquitofish, *Gambusia affinis,* in waters with suspended solids ranging from < 25 to 1400 mg/litre were reported by Wallen et al. (1957). Susceptibility of mosquito fish to ammonia was studied by Hemens (1966) who reported a 17-h LC_{50} of 1.3 mg NH_3/litre; he also observed that male fish were more susceptible than females. Rubin & Elmaraghy (1976, 1977) tested guppy, *Poecilia reticulata,* fry and reported 96-h LC_{50} values averaging 1.50 mg NH_3/litre; mature guppy males were more tolerant, with 100% survival for 96 h at concentrations of 0.17 - 1.58 mg NH_3/litre. LC_{50} values (96-h) of 0.15 and 0.20 mg NH_3/litre at pH 6.0, and of 0.52 and 2.13 mg NH_3/litre at pH 8.0, were reported by Stevenson (1977) for white perch, *Morone americana.* LC_{50} values (96-h) of 1.20 and 1.62 mg NH_3/litre for spotfin shiner, *Notropis spilopterus,* were reported by Rosage et al. (1979), and of 1.20 mg NH_3/litre for golden

shiner, *Notemigonus crysoleucas,* by Baird et al. (1979). Swigert & Spacie (1983) reported 96-h LC_{50} values of 0.72 mg NH_3/litre for golden shiner, 1.35 mg NH_3/litre for spotfin shiner, 1.25 mg NH_3/litre for steelcolour shiner, *Notropis whipplei,* and 1.72 mg NH_3/litre for stoneroller, *Campostoma anomalum.*

Jude (1973), Reinbold & Pescitelli (1982a), and McCormick et al. (1984) reported 96-h LC_{50} values ranging from 0.6 to 2.1 mg NH_3/litre for green sunfish, *Lepomis cyanellus.* In studies on the pumpkinseed sunfish, *Lepomis gibbosus,* by Jude (1973) and Thurston (1981), 96-h LC_{50} values ranged from 0.14 to 0.86 mg NH_3/litre. Mottled sculpin, *Cottus bairdi,* were tested by Thurston & Russo (1981), yielding a 96-h LC_{50} value of 1.39 mg NH_3/litre. An asymptotic (6-day) LC_{50} of 0.44 mg NH_3/litre was determined for rudd, *Scardinius erythropthalmus* (Ball, 1967).

Rao et al. (1975) reported a 96-h LC_{50} value for the common carp, *Cyprinus carpio,* of 1.1 mg NH_3/litre. Carp exposed to 0.24 mg NH_3/litre exhibited no adverse effects in 18 h (Vámos 1963). However, exposure to 0.67 mg NH_3/litre caused gasping and equilibrium disturbance within 18 min, frenetic swimming activity at 25 min, then sinking to the tank bottom after 60 min; after 75 min, the fish were placed in ammonia-free water and all revived. Kempinska (1968) reported a lethal concentration of 7.5 mg NH_3/litre for carp. Studies on the acute exposure of bitterling, *Rhodeus sericeus,* and carp to ammonium sulfate revealed minimum lethal concentrations of 0.76 mg NH_3/litre for bitterling and 1.4 mg NH_3/litre for carp (Malacea, 1966). Nehring (1963) reported survival times for carp at concentrations of 9.7 and 2.1 mg NH_3/litre of 2.4 and 6.0 h, respectively. The survival time for tench, *Tinca tinca,* was reported to be 20 - 24 h at 2.5 mg NH_3/litre by Danecker (1964). In a 24-h exposure of creek chub, *Semotilus atromaculatus,* to ammonium hydroxide solution, the "critical range" below which all test fish lived and above which all died was reported to be 0.26 - 1.2 mg NH_3/litre (Gillette et al., 1952).

In static exposures lasting 9 - 24 h, with a gradual increase in NH_3 content, mortalities occurred in oscar, *Astronutus ocellatus,* at 0.50 mg NH_3/litre (4%) to 1.8 mg/litre (100%) (Magalhaes Bastos, 1954). Tests on oscar of two different sizes showed no difference in susceptibility, in relation to size. A 72-h LC_{50} value of 2.85 mg NH_3/litre was reported by Redner & Stickney (1979) for blue tilapia, *Tilapia aurea.*

6.4.2.1. Saltwater fish

Very few acute toxicity data are available for salt-water fish species. Holland et al. (1960) reported the critical level for chinook salmon, *Oncorhynchus tshawytscha,* to be between 0.04 and 0.11 mg NH_3/litre and for coho salmon to be 0.134 mg NH_3/litre. A static test with coho salmon provided a 48-h LC_{50} value of 0.50 mg NH_3/litre (Katz & Pierro, 1967). Atlantic salmon smolts and yearling rainbow trout exposed for 24 h in 50 and 75% saltwater solutions exhibited similar sensitivities to ammonia (United Kingdom Ministry of Technology, 1963). Holt & Arnold (1983) reported a 96-h LC_{50} value of 0.47 mg NH_3/litre for red drum, *Sciaenops ocellatus.* LC_{50} values (96-h) of 1.2 - 2.4 mg NH_3/litre were reported by Venkataramiak et al. (1981) for striped mullet, *Mugil caphalus,* and 0.69 mg NH_3/litre for planehead filefish, *Monacanthus hispidus.*

6.4.3. Factors affecting acute toxicity

A number of factors can affect the toxicity of ammonia for aquatic organisms. These include the effects of pH, temperature, dissolved oxygen concentration, previous acclimatization to ammonia, fluctuating or intermittent exposures, carbon dioxide concentration, salinity, and the presence of other toxicants. Almost all studies of factors affecting ammonia toxicity have been carried out using only acute exposures.

6.4.3.1. pH

The toxicity for fish of aqueous solutions of ammonia and ammonium compounds has been attributed to the non-ionized (undissociated) ammonia present in the solution. The earliest reported thorough study of the pH dependence of ammonia toxicity was that of Chipman (1934), who concluded from studies on goldfish, amphipods, and cladocerans that ammonia toxicity was a function of pH and therefore of the concentration of undissociated ammonia in the solution. Downing & Merkens (1955) tested rainbow trout at different concentrations of ammonia at both pH 7 and pH 8. The results were consistent when ammonia concentration was expressed as NH_3. Tabata (1962) conducted 24-h tests on the toxicity of ammonia for *Daphnia* (species not specified) and guppy at different pH values and calculated the relative toxicity of NH_3/NH_4+ to be 48 for *Daphnia* and 190 for guppy (i.e., NH_3 is 190 times more toxic than NH_4+).

More recently, Robinson-Wilson & Seim (1975) studied the toxicity of ammonium chloride for juvenile coho salmon in flow-through bioassays within the pH range 7.0 - 8.5; the reported 96-h LC_{50} for NH_3 was approximately 60% less at pH 7.0 than at pH 8.5. The toxicity of ammonium chloride for larvae of prawn, *Macrobrachium rosenbergii,* was studied by Armstrong et al. (1978) in 6-day tests within the pH range 6.8 - 8.3 with test solutions being renewed every 24 h. The 96-h LC_{50} for NH_3 at pH 6.83 was approximately 70% less than that at pH 8.34. It was concluded that the toxicity of ammonia was not due solely to the NH_3 molecule and that in solutions of different pH, but equal NH_3 concentrations, survival was greatly reduced as NH_4+ levels increased. Tomasso et al. (1980) studied the toxicity of ammonia (NH_3) for channel catfish at pH values of 7, 8, and 9 and reported that 24-h LC_{50} values were significantly higher at pH 8 than at pH 7 or pH 9.

Thurston et al. (1981c) tested the toxicity of ammonia for rainbow trout and fathead minnows in 96-h flow-through tests at different pH levels within the range 6.5 - 9.0. Results showed that the toxicity of ammonia, in terms of NH_3, increased at lower pH values, and could also increase at higher pH values. It was concluded that NH_4+ exerts some measure of toxicity, and/or that increased H^+ concentration increases the toxicity of NH_3. Acute (96-h) exposures of green sunfish and smallmouth bass at 4 different pH levels over the range 6.5 - 8.7 showed that, for both species, NH_3 toxicity increased markedly with a decrease in pH, with LC_{50} values at the lowest pH tested (6.6 for sunfish, 6.5 for bass) being 3.6 (sunfish) and 2.6 (bass) times smaller than those at the highest pH (8.7) tested (McCormick et al., 1984; Broderius et al., in press).

It is concluded that NH_3 is more toxic for fish at lower pH values than within the pH range 7 - 9; the toxicity of NH_3 may increase again above this range.

6.4.3.2. Temperature

Information in the literature on the effects of temperature on ammonia toxicity is varied. The concentration of NH_3 increases with increasing temperature. Several researchers have reported an effect of temperature on the toxicity of the non-ionized ammonia species, independent of the effect of temperature on the aqueous ammonia equilibrium.

McCay & Vars (1931) reported that it took three times as long for the brown bullhead, *Ictalurus nebulosus,* to succumb to the toxicity of ammonia in water at 10 - 13 °C than at 26 °C. The pH of the tested water was not reported but with the probable range tested (pH 7 - 8), the percent NH_3 at the higher test temperature would have been approximately three times that at the mean lower temperature. The toxicity of ammonium chloride for goldfish, bluntnose minnow, *Pimephales notatus,* and the straw-coloured minnow or river shiner, *Notropis blennius,* was reported (Powers, 1920) to be greater at high temperatures than at low, but no consideration was given to the increase in the relative

concentration of NH_3 as the temperature increased. Herbert (1962) suggested that the effects of temperature on the susceptibility of rainbow trout to NH_3 toxicity was only slightly, if at all, affected by temperature change. In studies on striped bass, *Morone saxtilis,* and stickleback, *Gasterosteus aculeatus,* Hazel et al. (1971) found a slight difference in toxicity between 15° and 23 °C in fresh water, with both fish species being slightly more resistant at the lower temperature.

However, there are other studies in which the toxicity of NH_3 decreased with increasing temperature over the ranges studied. The toxicity of NH_3 for rainbow trout has been reported to be much higher at 5 °C than at 18 °C (United Kingdom Ministry of Technology, 1968). Brown (1968) reported that the 48-h LC_{50} for rainbow trout increased with increase in temperature over the range 3 °C - 18 °C; the reported increase in tolerance between ~12 °C - ~18 °C was considerably less than that between ~3 °C - ~12 °C. A relationship between temperature and 96-h LC_{50} was reported for rainbow trout over the temperature range 12 °C - 19 °C with ammonia toxicity decreasing with increasing temperature (Thurston & Russo, 1983).

Thurston et al. (1983) reported that the acute toxicity of NH_3 for fathead minnows decreased with a rise in temperature over the range 12 °C - 22 °C. Bluegill and fathead minnow were tested at low and high temperatures of 4.0 °C - 4.6 °C and 23.9 °C - 25.2 °C, respectively, and rainbow trout were tested at 3 °C and 14 °C (Reinbold & Pescitelli, 1982b). All three species were more sensitive to NH_3 at the low temperatures, with toxicity being 1.5 - 5 times higher in the colder water. Bluegill appeared to be the most sensitive of the three species to the effects of low temperature on ammonia toxicity. Colt & Tchobanoglous (1976) reported that the toxicity of NH_3 for channel catfish decreased with increasing temperature over the range 22 °C - 30 °C. LC_{50} values for bluegill, channel catfish, and largemouth bass at 28 °C - 30 °C were approximately twice those at 22 °C (Roseboom & Richey, 1977). An effluent containing ammonia as the principal toxic component showed a marked decrease in toxicity for channel catfish over the temperature range 4.6 °C - 21.3 °C (Cary, 1976).

Lloyd & Orr (1969) investigated the effects of temperature (range 10 - 20 °C) on urine flow rates in rainbow trout exposed to 0.30 mg NH_3/litre, and did not find any apparent temperature effect on the total diuretic response of the fish, though the relative

increase in urine production was less at higher temperatures. From a study of the behavioural response of bluegill to gradients of ammonium chloride, it was hypothesized that low temperatures increased the sensitivity of the bluegill and interfered with the ability, either to detect ammonia after a certain period of exposure, or, to compensate behaviourally for physiological stress caused by ammonia gradients (Lubinski, 1979; Lubinski et al., 1980).

The European Inland Fisheries Advisory Commission (1970) has stated that, at temperatures below 5 °C, the toxic effects of non-ionized ammonia may be greater than at above 5 °C, though the basis for this is not clearly documented. The evidence that temperature, independent of its role in the aqueous ammonia equilibrium, affects the toxicity of NH_3 for fish argues for further consideration of the temperature/ammonia toxicity relationship.

6.4.3.3. Salinity

Herbert & Shurben (1965) reported that the resistance of yearling rainbow trout to ammonium chloride increased with increasing salinity up to levels of 30 - 40% sea water; above this level, resistance appeared to decrease. Fingerling coho salmon were tested at salinity levels of 20 - 30 parts per thousand (57 - 86% salt water), and it was found that the toxicity of an ammonia-ammonium waste increased as salinity increased (Katz & Pierro, 1967). These findings are in agreement, at the levels tested, with those of Herbert & Shurben (1965). Atlantic salmon were exposed to ammonium chloride solutions for 24 h under both fresh-water and 30% salt-water conditions; LC_{50} values were 0.15 and 0.3 mg NH_3/litre, respectively, in the 2 different waters (Alabaster et al., 1979). Harader & Allen (1983) reported that the resistance to ammonia of chinook salmon parr increased by about 500%, as salinity increased to almost 30% sea water, but declined as salinity increased beyond that.

There is a slight decrease in the NH_3 fraction of total ammonia as ionic strength increases in dilute saline solutions, but the relative changes in NH_3 toxicity, as salinity increases, are more directly attributable to changes in the rate of exchange of NH_3 and NH_4+ across the fish gill membranes.

6.4.3.4. Dissolved oxygen

A decrease in dissolved oxygen concentration in the water can increase ammonia toxicity. There is a reduction in fish blood oxygen-carrying capacity following ammonia exposure (Brockway, 1950; Danecker, 1964; Reichenback-Klinke, 1967; Körting, 1969a,b; Waluga & Flis, 1971). Hypoxia would further exacerbate problems of oxygen delivery and could lead to the early demise of the fish.

Vámos & Tasnádi (1967) observed deaths of carp in ponds at ammonia concentrations lower than would normally be lethal, and attributed this to periodic low concentrations of oxygen. On the basis of research in warm-water (20 °C - 22 °C) fish ponds, Selesi & Vámos (1976) projected a "lethal line", relating acute ammonia toxicity and dissolved oxygen, below which carp died. The line ran between 0.2 mg NH_3/litre at 5 mg dissolved oxygen/litre and 1.2 mg NH_3/litre at 10 mg dissolved oxygen/litre. Thurston et al. (1983) compared the acute toxicity of ammonia for fathead minnows at reduced and normal dissolved oxygen concentrations; seven 96-h tests were conducted within the range 2.6 - 4.9 mg dissolved oxygen/ litre, and 3 between 8.7 and 8.9 mg/litre. There was a

slight positive trend between 96-h LC_{50} values and dissolved oxygen, though it was not shown to be statistically significant. Atlantic salmon smolts were tested in both fresh water and 30% salt water at 9.6 - 9.5 and 3.5 - 3.1 mg dissolved oxygen/litre. The reported 24-h LC_{50} values at the higher oxygen concentrations were about twice those at the lower (Alabaster et al., 1979).

Several studies have been reported on rainbow trout. Allan (1955) reported that below 0.12 mg NH_3/litre and at about 30% oxygen saturation, the median survival time was greater than 24 h, but at the same concentration with oxygen saturation below 30%, the median survival time was less than 24 h. In studies by Downing & Merkens (1955), fingerling rainbow trout were tested at 3 different concentrations of NH_3 at 5 different levels of dissolved oxygen. In tests lasting up to 17 h, decreasing the oxygen level from 8.5 to 1.5 mg/litre shortened the period of survival at all ammonia concentrations, and a decrease in survival time produced by a given decrease in oxygen was greatest at the lowest concentration of NH_3. Merkens & Downing (1957), in tests lasting up to 13 days, also reported that the effects of low concentrations of dissolved oxygen on the survival of rainbow trout were more pronounced at low concentrations of NH_3. Ammonia (NH_3) was found to be up to 2.5 times more toxic when the dissolved oxygen concentration was reduced from 100 to about 40% saturation (Lloyd, 1961). It was reported by Danecker (1964) that the toxicity of ammonia increased rapidly when the oxygen concentration decreased below two-thirds of the saturation value. Thurston et al. (1981b) conducted 15, 96-h acute toxicity tests on rainbow trout over the dissolved oxygen range 2.6 - 8.6 mg/litre. A positive linear correlation between 96-h LC_{50} and dissolved oxygen was reported over the entire range tested.

When rainbow trout were treated in a channel receiving sewage discharge containing 0.05 - 0.06 mg NH_3/litre, it was found that, at 25 - 35% dissolved oxygen saturation, more than 50% of the fish died within 24 h, compared with 50% mortality of test fish in the laboratory, at 15% dissolved oxygen saturation (Herbert, 1956). The difference was attributed to unfavourable water conditions below the sewage outflow, including ammonia, which increased the sensitivity of the fish to the lack of oxygen.

6.4.3.5. Carbon dioxide

An increase in carbon dioxide (CO_2) concentrations up to 30 mg/litre decreased total ammonia toxicity (Alabaster & Herbert, 1954; Allan et al., 1958). Carbon dioxide causes a decrease in pH, thereby decreasing the proportion of non-ionized ammonia in solution. However, Lloyd & Herbert (1960) found that, though total ammonia toxicity was reduced at elevated CO_2 levels, the inverse was true when considering non-ionized ammonia alone; more NH_3 was required in low CO_2, high pH water to exert the toxic effect seen in fish in high CO_2, low pH water. The explanation presented by Lloyd & Herbert (1960) for the decreased toxicity of NH_3 in low CO_2 water was that CO_2 excretion across the gills would reduce the pH and, therefore, the NH_3 concentration, in water flowing over the gills. A basic flaw in this hypothesis has been discussed by Broderius et al. (1977). Carbon dioxide will only form protons very slowly in water at the tested temperature. The uncatalysed CO_2 hydration reaction has a half-time of seconds or even min (e.g., at pH 8: 25 seconds at 25 °C; 300 seconds at 0 °C) (Kern 1960), and water does not remain in the opercular cavity for more

than a few seconds, and at the surface of a gill lamella for about 0.5 - 1 second (Randall, 1970; Cameron, 1979). Thus, the liberation of CO_2 across the gills will have little, if any, effect on water pH or NH_3 levels and the NH_3 gradient across the gills between water and blood.

6.4.3.6. Prior acclimatization to ammonia

The question of whether fishes can acquire an increased tolerance to ammonia by acclimatization to low ammonia concentrations is an important one. If fish were able to develop such tolerance, they might be able to survive what would otherwise be lethal ammonia concentrations.

Observations by McCay & Vars (1931) indicated that brown bullheads subjected to several successive exposures to ammonia, alternating with recovery in fresh water, did not acquire tolerance. However, a number of research workers have reported that previous exposure of fish to low concentrations of ammonia increases their resistance to lethal concentrations. Vámos (1963) reported that carp exposed to 0.67 or 0.52 mg NH_3/litre for 75 min, then transferred to fresh water for 12 h, followed by a solution containing 0.7 mg NH_3/litre, exhibited symptoms of ammonia toxicity in 60 - 85 min, whereas control fish, exposed initially to 0.7 mg/litre NH_3, developed symptoms within 20 min. Blue tilapia acclimatized for 35 days to 0.52 - 0.64 mg NH_3/litre subsequently survived 48 h at 4.1 mg/litre, compared with the 48-h value for unacclimatized fish of 2.9 mg/litre (Redner & Stickney, 1979). Malacea (1968) studied the effects on bitterling of acclimatization to ammonium sulfate solutions. A group of 10 fish was held in an acclimatization solution of 0.26 mg NH_3/litre for 94 h, after which the fish were exposed to a 5.1 mg NH_3/litre solution for 240 min. A control group of 10 bitterling received identical treatment, except that the acclimatization aquarium did not contain added $(NH_4)_2SO_4$. The ratio of the mean survival times of "adapted" to "unadapted" fish was 1:13, indicating a slightly higher ammonia tolerance for the adapted fish.

Schulze-Wiehenbrauck (1976) subjected 2 groups of rainbow trout (mean weights 56 g and 110 g) that had been held for at least 3 weeks at sublethal ammonia concentrations, to lethal ammonia concentrations. In the study on the 110-g fish, the acclimatization concentrations were 0.007, 0.131, and 0.167 mg NH_3/litre. The fish were then subjected for 8.5 h to concentrations of 0.45, 0.42 and 0.47 mg NH_3/litre, respectively. Fish from the 2 higher sublethal concentrations showed 100% survival after 8.5 h in the 0.42 and 0.47 mg NH_3/litre solutions, whereas fish from the 0.007 mg NH_3/litre concentration showed only 50% survival in 0.45 mg NH_3/litre. In the study on the 56-g fish,

the acclimatization concentrations were 0.004 mg NH_3/litre and 0.159 mg NH_3/litre; these fish were placed for 10.25 h in NH_3 concentrations of 0.515 and 0.523 mg/litre, respectively. There was 100% survival in the acclimatized fish, and 85% survival in the fish acclimatized to 0.004 mg/litre. The results of these studies showed an increase in resistance of trout to high ammonia levels after prior exposure to sublethal ammonia levels.

Alabaster et al. (1979) determined 24-h LC_{50} values of NH_3 for Atlantic salmon smolts under reduced dissolved oxygen test conditions. Fish acclimatized to ammonia before oxygen reduction had LC_{50} values 38% and 79% higher than fish without prior ammonia acclimatization.

In studies by Brown et al. (1969), rainbow trout were tested by moving back and forth between tanks in which the ammonia concentrations were 0.5 and 2.5 times a previously determined 48-h LC_{50} value. If fish were transferred on an hourly basis, the median period of survival for the fluctuating exposure was reported to be the same as that for constant exposure (> 700 min). When the fish were transferred at 2-h intervals, the median survival time for the fluctuating exposure was reported to be less (370 min), indicating that the toxic effects from exposure to the fluctuating concentrations of ammonia were greater than those from exposure to the constant concentration. Thurston et al. (1981a) conducted acute toxicity tests in which rainbow trout and cutthroat trout were exposed to short-term cyclical fluctuations of ammonia. Companion tests were conducted in which test fish were subjected to ammonia at constant concentrations. The LC_{50} values for both average and peak concentrations of ammonia for the fluctuating concentration tests were compared with the LC_{50} values for the constant concentration tests. Comparison of total exposures showed that fish were more tolerant to constant, than to fluctuating concentrations of ammonia. Fish subjected to fluctuating concentrations of ammonia at levels below those acutely toxic were better able to withstand subsequent exposure to high fluctuating concentrations than unacclimatized fish. There is reasonable evidence that fish with a history of prior exposure to sublethal concentrations of ammonia are better able to withstand an acutely lethal concentration for a period of hours and possibly days. Limited data on fluctuating exposures indicate that fish are more susceptible to fluctuating than to constant exposure with the same average NH_3 concentrations.

6.4.4. Fish: chronic toxicity

"Full-chronic" tests cover the entire life cycle of the test animal, beginning at a given stage of development of one generation (frequently as the fertilized egg) and continuing through to this same stage in the next generation. Common end-points for measuring toxicity are survival, growth, and reproductive success, though recent research on ammonia toxicity for fish has demonstrated the desirability of also conducting histological examinations.

"Partial-chronic" tests on fish most frequently cover a period of 30 days or longer, from the egg incubation stage to the free-swimming stage; for many toxins it has been demonstrated that these stages are the most sensitive. However, in the case of ammonia it has been demonstrated that older, mature rainbow trout, *Salmo gairdneri,* are potentially as susceptible to the effects of ammonia as newly-hatched larvae.

Long-term ammonia exposure of fishes, including complete life-cycle tests on rainbow trout and fathead minnows with several end-points, including effects on spawning and egg incubation, growth, survival and tissues, have been studied. The effects of prolonged exposure to ammonia (up to 61 days) on the early life stages of of pink salmon were studied by Rice & Bailey (1980). Three series of exposures were carried out, beginning at selected times after hatching. These were for 21 days prior to completion of yolk absorption, for 40 days up to 21 days before yolk absorption, and for 61 days up to yolk absorption. All test fish were sampled for size when the controls had completed yolk absorption. Test concentrations ranged from zero up to 0.004 mg NH_3/litre. For fry at the highest concentration of 0.004 mg NH_3/litre, significant decreases in weight were observed in all 3 exposed groups. At a concentration of 0.0024 mg NH_3/litre, the groups of fry exposed for

40 and 61 days were significantly smaller, whereas a concentration of 0.0012 mg/litre had no significant effect on growth. Effects were consistently more marked for the 61-day-exposed fish.

In a 3-generation, 5-year laboratory study, rainbow trout exposed for 5 months to concentrations of ammonia ranging from 0.01 to 0.07 mg NH_3/litre, spawned of their own volition. There was no correlation between ammonia concentration and numbers of egg lots spawned, total numbers of eggs produced, or numbers of eggs subsequently hatched. Parental fish were exposed for 11 months, the first filial generation (F_1) for 4 years, and the second filial generation (F_2) for 5 months. Pathological lesions were observed in both parental and F_1 fish, when ammonia concentrations reached and exceeded 0.04 mg NH_3/litre. Measurements of blood-ammonia concentrations in 4-year-old F_1 fish showed an increase when test water concentrations reached or exceeded 0.04 mg NH_3/litre. The F_1 fish exposed for 52 months from day of hatching showed no relationship between growth and concentration at 10, 15, 21, and 52 months (Thurston et al., 1984b).

Burkhalter & Kaya (1977) tested ammonia at concentrations ranging from 0.06 to 0.45 mg/litre on fertilized eggs and the resultant sac fry of rainbow trout. Eggs were incubated at 12 °C for 25 days in one test and at 10 °C for 33 days in another; fry were maintained for 42 days. No concentration response was seen in egg mortality or incubation time in either test. Retardation in early growth and development occurred at 0.06 mg NH_3/litre, the lowest concentration tested. Fish exposed to 0.12 mg NH_3/litre required 1 week longer than the controls to achieve a free-swimming state; fish at 0.34 and 0.45 mg NH_3/litre did not achieve a free-swimming state during a 42-day test period. A 21-day LC_{50} value of 0.30 mg NH_3/litre was obtained. For sac fry exposed for 42 days after hatching, hypertrophy of secondary gill lamellae epithelium occurred at 0.23 mg NH_3/litre, and karyolysis and karyorrhexis in the secondary gill lamellae were observed after 28 days at 0.34 mg NH_3/litre and higher.

Calamari et al. (1977, 1981) exposed rainbow trout to ammonium chloride solutions for 71 days, beginning 1 day after fertilization and ending when fry had been feeding for 30 days. A 72-day LC_{50} of 0.056 mg NH_3/litre was calculated; 23% mortality occurred at a concentration of 0.025 mg/litre. Examination of 986 rainbow trout embryos at the hatching stage after exposure to concentrations of 0.010 - 0.193 mg NH_3/litre for 24 days showed an increase in gross malformations with increasing ammonia concentration. The deformities observed were various degrees of curvature from the median body axis, and various kinds of malformations in the head region with a number of cases of double heads. At the highest concentration tested, 0.193 mg NH_3/litre, 60% of the observed fish were malformed. Microscopic examination, at hatching, of 128 larvae from the same exposure showed abnormalities of the epidermis and pronephros, which were correlated with ammonia concentrations. The epidermis was thickened with an irregular arrangement of the various layers of cells and an increase in the number and dimensions of mucous cells. The pronephros showed widespread vacuolization of the tubule cells, together with a thickening of the wall. Increasing abnormalities were observed after exposure to concentrations exceeding 0.025 mg NH_3/litre for the epidermis and 0.063 mg/litre for the pronephros.

Four 4-week-old rainbow trout fry were exposed for 30 days to concentrations of ammonia (reported graphically) ranging from

~0.06 to 0.31 mg NH_3/litre. Growth rate at ~0.06 mg NH_3/litre was comparable with that of controls, but, above ~0.10 mg NH_3/litre, growth rate decreased, in correlation with increased NH_3 concentration. Survival at 0.32 mg NH_3/litre was 70% of that of the controls (Broderius & Smith, 1979). Schulze-Wiehenbrauck (1976) tested juvenile rainbow trout of different sizes, for periods of time ranging from 2 to 7 weeks, and at ammonia concentrations ranging from 0.012 to 0.17 mg/litre. He concluded that a concentration of 0.05 mg NH_3/litre caused a slight decrease in growth during the first 14-day interval in non-acclimatized fish, but that the decrease was completely compensated for in the next growth interval. Exposure to 0.13 mg NH_3/litre (apparently for 3 or 4 weeks) did not affect growth, food consumption, or food conversion.

Young rainbow trout were reared in 3 concentrations of ammonia (averaging 0.006, 0.012, and 0.017 mg/litre) for a period of 1 year. At 4 months, there was no significant difference in fish growth at the 3 concentrations. At 11 months, there was a difference with the fish at 0.012 and 0.017 mg NH_3/litre, which weighed 9% and 38% less, respectively, than the fish at 0.006 mg NH_3/litre. Microscopic examination of tissues from fish exposed to the highest concentration, examined at 6, 9, and 12 months, showed severe pathological changes in gill and liver tissues. Gills showed extensive proliferation of the epithelium, which resulted in severe fusion of gill lamellae preventing normal respiration. Livers showed reduced glycogen storage and scattered areas of dead cells; these became more extensive with increase in exposure time (Smith, 1972; Smith & Piper, 1975).

Rainbow trout were exposed for 3 months to concentrations of 0.069, 0.14, and 0.28 mg NH_3/litre. The cumulative mortality of a control group (0.005 mg NH_3/litre) was ~2%; cumulative mortality at 0.069 and 0.14 mg/litre was ~5%, and that at 0.28 mg/litre was ~15% (United Kingdom Ministry of Technology, 1968). Reichenbach-Klinke (1967) performed a series of 1-week tests on 240 fish of 9 species (including rainbow trout, goldfish, northern pike, *Esox lucius,* carp, and tench) at concentrations of 0.1 - 0.4 mg NH_3/litre. Swelling of, and diminution of the number of, red blood cells, inflammation, and hyperplasia were observed. Irreversible blood damage occurred in rainbow trout fry at concentrations above 0.27 mg NH_3/litre. Low NH_3 concentrations also inhibited the growth of young trout and lessened their resistance to disease.

In rainbow trout exposed to 0.30 to 0.36 mg NH_3/litre, 81% mortality occurred over the 36-day duration of the test, with most deaths occurring between days 14 and 21. Microscopic examination of the gills revealed some thickening of the lamellar epithelium and an increased mucous production. The most characteristic feature was a large proportion of swollen, rounded secondary lamellae in which the pillar system was broken down and the epithelium enclosed a disorganized mass of pillar cells and erythrocytes. Gill hyperplasia was not a characteristic observation (Smart, 1976).

In rainbow trout exposed to < 0.0005 or 0.005 mg NH_3/litre for 8 weeks, examination of the gill lamellae of fish from the lower concentration showed them to be long and slender with no significant pathology. Fish exposed to 0.005 mg NH_3/litre had shorter and thicker gill lamellae with bulbous ends, and some consolidation of lamellae was noticed. Many filaments showed a definite hyperplasia of the epithelial layer, evidenced by an

increase in the number of cell nuclei (Fromm, 1970).

Thurston et al. (1978) studied the toxicity of ammonia for cutthroat trout fry in tests that lasted up to 36 days. Results of duplicate tests on 1-g fish showed 29- and 36-day LC_{50} values of 0.56 mg NH_3/litre. Duplicate tests on 3-g fish provided 29-day LC_{50} values of 0.37 and 0.34 mg NH_3/litre, slightly less than those of the 1-g fish. The heart, gastrointestinal tract, and thymus of cutthroat trout fry exposed to 0.34 mg NH_3/litre for 29 days were comparable with those of control fish, but the gills and kidneys showed degenerative changes. The gills showed hypertrophy of epithelium, some necrosis of epithelial cells, and separation of epithelium due to oedema. The kidneys had mild hydropic degeneration and accumulation of hyaline droplets in the renal tubular epithelium. Reduced vacuolation was observed in livers.

Samylin (1969) studied the effects of ammonium carbonate on the early stages of development of Atlantic salmon. The first set of studies, at 13 °C, lasted 53 days and was conducted within the range 0.001 to > 6.6 mg NH_3/litre beginning with the "formed embryo" stage. Accelerated hatching was observed with increasing $(NH_4)_2CO_3$ concentrations, but concentrations of $\geq$ 0.16 mg NH_3/litre were lethal for emerging larvae within 12 - 36 h. Because $(NH_4)_2CO_3$ was used as the toxin, the pH in the test aquaria increased from 6.7 to 7.6 with increasing NH_3 concentration. Growth inhibition was observed at 0.07 mg NH_3/litre. Tissue changes were observed in eyes, brains, fins, and blood of Atlantic salmon embryos and larvae exposed to concentrations ranging from 0.16 to > 6.6 mg NH_3/litre, with more marked changes at higher ammonia concentrations. The effects observed included erosion of membranes of the eyes and shedding of the crystalline lens, dilatation of blood vessels in the liver and brain, accumulation of blood in the occipital region and in the intestines. Reaction to light and mechanical stimulation gradually disappeared with increased ammonia concentration, and the heart rate slowed. Morphological differences in development between experimental and control larvae were observed from the tenth day of exposure, including a lag in yolk resorption, decrease in growth of the skin fold, and contraction of skin pigment cells causing the skin colour to become paler than it was after hatching. At concentrations up to 0.07 mg NH_3/litre, no significant morphological differences were observed.

A second series of studies, at 16.5 °C, was carried out in the 0.001 - 0.32 mg NH_3/litre concentration range, beginning with larval salmon (Samylin 1969). Concentrations of $\geq$ 0.21 mg/litre were lethal and caused weight loss in fry; 0.001 - 0.09 mg NH_3/litre caused a decrease in weight gain, though there were no differences in feeding activity, behaviour, or development at these concentrations compared with controls. Dissolved oxygen concentrations in this second series of studies dropped as low as 3.5 mg/litre.

Burrows (1964) tested fingerling chinook salmon for 6 weeks in outdoor water channels into which ammonium hydroxide was introduced. Two studies were conducted, one at 6.1 °C and the other at 13.9 °C, both at pH 7.8. The fish were then maintained in fresh water for an additional 3 weeks. A recalculation of the reported non-ionized ammonia concentrations, based on more recent aqueous ammonia equilibrium tables, shows that the concentrations at 6.1 °C were 0.003 - 0.006 mg NH_3/litre and, at 13.9 °C, were 0.005 - 0.011 mg NH_3/litre. At both temperatures, and at all ammonia concentrations, some fish showed excessive proliferation and clubbing of the gill filaments. The proliferation was

progressive for the first 4 weeks, after which no measurable increase was observed. After 3 weeks in fresh water, examination of fish exposed at 6.1 °C indicated that recovery from the extensive proliferation had not taken place. In the study on larger fish at 13.9 °C, a marked recovery from hyperplasia was noted after the 3 weeks in fresh water. In the first study, the proliferated areas had consolidated; in the second, they had not. It was postulated that continuous ammonia exposure is a precursor of bacterial gill disease.

Duplicate groups (90 fish each) of hatchery-reared coho salmon were exposed for 91 days to "river-water" solutions of ammonium chloride at concentrations of 0.019 - 0.33 mg NH_3/litre. Control groups were reared at 0.002 mg/litre. Haemoglobin content and haematocrit readings were slightly, but significantly, reduced in fish exposed to the highest concentration tested, and there was also a greater percentage of immature erythrocytes. Blood-ammonia and -urea concentrations were not significantly different after 91 days, regardless of the concentration of ammonia to which the fish were exposed (Buckley et al., 1979). Rankin (1979) exposed embryos of sockeye salmon, *Oncorhynchus nerka,* to ammonia from fertilization to hatching. Total embryo lethality occurred at concentrations of 0.49 - 4.9 mg NH_3/litre. The times required to achieve 50% mortality at these concentrations were 40 - 26 days. Mortality of the embryos exposed to 0.12 mg NH_3/litre was 30%, and time to 50% mortality was 66 days.

Two full life-cycle ammonia toxicity tests, each lasting approximately 1 year, were conducted on fathead minnows (Thurston et al., in press). These tests began with newly hatched fry and were continued through their growth, maturation and spawning stages; progeny were exposed from hatching through growth to 60 days of age. While no statistically-significant differences were observed in survival, growth, egg production, and egg viability, at concentrations up to 0.4 mg NH_3/litre, effects were seen at 0.4 mg NH_3/litre.

Tissues from fathead minnows subjected to prolonged (up to 304 days) ammonia exposure were examined (Smith, 1984). Growths, some massive, were observed on the heads of several fish exposed to concentrations of 1.25 or 2.17 mg NH_3/litre, and swollen darkened areas were observed on the heads of several fish exposed to 0.639 - 1.07 mg NH_3/litre. Thurston et al. (in press) also reported lesions at concentrations below those at which other effects were observed. Brain lesions were common at concentrations of 0.21 mg NH_3/litre and higher. Grossly and histologically, the severity of the lesions, which varied from mild to severe, was positively correlated with ammonia concentration. The lesions appeared to be of a cell type originating from the meninx primativa covering the brain. The hyperplastic tissue often completely surrounded the brain but was not observed around the spinal cord.

An early life-stage test initiated at the blastula stage of embryogenesis and extending through 39 days post-hatching was conducted on green sunfish (McCormick et al., 1984). Retardation of growth was found in green sunfish exposed from embryo through juvenile life stages to concentrations of 0.489 mg NH_3/litre or more, but not at 0.219 mg NH_3/litre. In a long-term test on green sunfish, Jude (1973) reported that, at levels higher than 0.17 mg NH_3/litre, mean fish weight increased less rapidly than that of the controls on the 4 days following the introduction of ammonia. Thereafter, fish exposed to 0.26 and 0.35 mg NH_3/litre grew at an increasing rate, while fish exposed to 0.68 and 0.64 mg NH_3/litre remained the same for 12 days before increases in growth occurred.

Four simultaneous early life-stage ammonia tests with smallmouth bass were carried out at 4 different pH levels, ranging from 6.6 to 8.7, in order to examine the effect of pH on chronic ammonia toxicity. Exposure to ammonium chloride solutions began on 2- to 3-day old embryos and lasted for 32 days. The end-point observed was growth, and ammonia was found to have a greater effect on growth at lower pH levels than at high. Concentrations found to retard growth ranged from 0.056 mg/litre at pH 6.60, to 0.865 mg/litre at pH 8.68 (Broderius et al., in press).

In early life-stage tests (29 - 31 days' exposure) on channel catfish and white sucker, no significant effects on percent hatch or larval survival were observed for channel catfish exposed to ammonium chloride at concentrations as high as 0.583 mg NH_3/litre and for white sucker at concentrations as high as 0.239 mg NH_3/litre. Significant retardation of growth, however, occurred in channel catfish at concentrations of 0.392 mg NH_3/litre or more and in white sucker at 0.070 mg NH_3/litre and higher. A delay in time to swim-up stage was also observed for both species at elevated (0.06 - 0.07 mg/litre) ammonia concentrations (Reinbold & Pescitelli, 1982a).

In cultured channel catfish fingerlings, exposed for periods of approximately 1 month to concentrations of 0.01 - 0.16 mg NH_3/litre, growth at 0.01 and 0.07 mg NH_3/litre was not significantly different from that of control fish, but growth retardation at 0.15 and 0.16 mg NH_3/litre was statistically significant (Robinette, 1976). Colt (1978) and Colt & Tchobanoglous (1978) reported retardation of growth of juvenile channel catfish during a 31-day period of exposure to concentrations ranging from 0.058 to 1.2 mg NH_3/litre. Growth rate was reduced by 50% at 0.63 mg NH_3/litre, and no growth occurred at 1.2 mg NH_3/litre.

6.5. Wild and Domesticated Animals

6.5.1. Wildlife

Although ammonia has been known to be toxic for nearly a century (Hahn et al., 1983), studies describing the toxicological effects of ammonia on wildlife are very limited. Normally, atmospheric ammonia does not appear to be a problem for wild animals, but concentrations of ammonia could reach harmful levels in accidents during transport near forests and remote areas. NRC (1979) has reported 2 types of observations in relation to this topic: (a) the use of anhydrous ammonia to exterminate wild birds and mice in farm buildings; and (b) the tolerance of bats to atmospheric ammonia.

The use of anhydrous ammonia has been recommended for exterminating wild birds and mice from farm buildings by Day et al. (1965). The technique is simple, economical, and does not leave any harmful residue. The farm buildings, after removal of the livestock, were sealed and treated with anhydrous ammonia at 1600 mg/m^3 (2285 ppm) for 7 min and then reopened. Ammonia fumes were fatal for the wild inhabitants, particularly for wild birds.

Within 0.5 h, dead starlings, sparrows, pigeons, and mice were removed from the barns. Farm animals were allowed to enter the barns within 1 h of their reopening. According to laboratory studies, the mouse appears to be more sensitive than other animal species such as the rat, rabbit, and guinea-pig. When mice were exposed for 10 min to ammonia at 6140 - 9060 mg/m^3 (8770 - 12 940

ppm), death with convulsion began after 5 min of exposure, and over 50% of the mice died before the study was completed. The surviving animals appeared to recover rapidly, but another 4% died between the 6th and 10th days after exposure (Underwriters Laboratories, 1933).

Large colonies of Guano bats *(Tadarida brasiliensis)* frequently inhabit caves or other areas, producing large amounts of guano, which, on bacterial decomposition, results in a very high concentration of ammonia in the atmosphere. Although high ammonia concentrations, together with high relative humidity in caves, discoloured the pelage of bats (Eads et al., 1955; Constantine, 1958; Mitchell, 1964), no other adverse physiological effects were observed in these mammals. This apparent adaptation to inhaled ammonia prompted laboratory studies relating to the physiological mechanisms involved in ammonia tolerance in different species of bats (Mitchell, 1963; Studier, 1966; Studier et al., 1967).

California leaf-nosed bats *(Macrotus californicus)* can tolerate exposure to 2100 mg/m^3 (3000 ppm) for up to 9 h (Mitchell, 1963).

Ammonia toxicity at lethal doses was manifested by corrosion of the skin and mucous membranes, pulmonary oedema, and distinct visceral damage. The blood-non-protein nitrogen in the exposed bats was significantly elevated without any increase in urinary-urea or -ammonia.

Studier et al. (1967) studied the effects of increasing concentrations of atmospheric ammonia on ammonia tolerance and metabolic rates in rats, mice, and 3 species of bat. Rats, mice, and 2 species of bat *(Myotis lucifugus* and *Eptesicus fuscus)* tended to show increased oxygen utilization, when exposed to increased ammonia levels. However, the guano bat *(Tadarida brasiliensis)* exhibited a large decrease in oxygen utilization with increasing ammonia concentration (i.e., 74% depression) when exposed to air containing 4900 mg/m^3 (7000 ppm) of gaseous ammonia. Earlier, Studier (1966) had shown that ~35% of gaseous ammonia filtered through the mucous linings in the respiratory passage in guano bats, when they were exposed to 2100 mg/m^3 (3000 ppm) of ammonia. There was no change in their normal blood pH during exposures to high ammonia concentrations. The animals, however, exhaled measurable amounts of ammonia when transferred to normal air.

6.5.2. Domesticated animals

6.5.2.1. Oral exposure

(a) *Ruminants*

The use of urea as a partial source of nitrogen in ruminant nutrition is limited by its toxicity, which results from its metabolism to ammonia.

The toxic effects of urea in ruminants are related to a high ammonia content in the blood. Urea itself is not as toxic as the ammonia, which is rapidly released in the rumen by the action of bacterial urease (EC 3.5.1.5) on ingested urea (Bloomfield et al., 1960). The absorption of this excess ammonia has been shown to depend on the pH of the ruminal contents.

Hogan (1961) examined the effects of pH on the absorption of ammonia from the rumen in sheep. When an ammonia-containing buffer

at pH 6.5 was placed in the rumen, absorption increased with the concentration gradient. At a pH of 4.5, however, the concentration of ammonia in the rumen did not affect the absorption across the epithelium. The net loss of ammonia-nitrogen from the rumen at pH 6.5 was more than 3 times the loss at a pH of 4.5.

Additional support for the effect of pH on ammonia absorption across the ruminal epithelium in sheep has been presented by Bloomfield et al., (1962). As the pH of the ruminal contents increased from 6.21 to 6.45, no ammonia was absorbed; however, as the pH increased to 7.59, the absorption rate was 16 mmol/litre per h. One sheep with a ruminal pH of 7.7 died of ammonia toxicity within 30 min. These data support the hypothesis that the non-ionized ammonia, which increases at higher pH, penetrates the lipid layers of the ruminal epithelium more effectively than the charged ammonium ion (Coombe et al., 1960).

Toxic signs become apparent as the blood-ammonia-nitrogen increases to 10 mg/litre; tetanic spasms occur between 10 and 20 mg/litre and are followed by death (Repp et al., 1955; McBarron & McInnes, 1968; Kirkpatrick et al., 1972, 1973; Webb et al., 1972). Wilson et al. (1968a) attributed the cause of death to the cardiotoxic effects of ammonia produced from the urea. However, Singer & McCarty (1971) observed that only one sheep died of ventricular fibrillation and the remainder of respiratory failure. More recently, Edjtehadi et al. (1978) reported the arrest of respiration, and not cardiovascular collapse, as the cause of death in sheep.

Certain aspects of the blood chemistry have been described for sheep with urea poisoning (Kirkpatrick et al., 1973; Edjtehadi et al., 1978). In general, during the initial stages of urea toxicosis, an alkalosis is induced, followed by systemic acidosis due to hyperventilation prior to death (Edjtehadi et al., 1978). In addition to increases in blood-ammonia and blood-urea levels, there is a marked increase in the blood-glucose level, with no change in ketone concentrations in the body (Singer & McCarty, 1971). The following changes have been recorded at death: red-cell count and haemoglobin concentration increased by 7.9%; white-cell count decreased by 27.5%; and packed-cell volume increased by 11.4%. Mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration were not substantially changed (Kirkpatrick et al., 1973).

Pathological effects of ammonia toxicity in sheep have been described by Singer & McCarty (1971). The changes were similar when sheep received intraruminal injections of ammonium chloride, ammonium sulfate, or a mixture of ammonium chloride, carbonate, phosphate, and sulfate. General passive hyperaemia and numerous petechial and ecchymotic haemorrhages in the musculature, heart, thymus, and lungs were found. The lungs were distended and severely congested. On microscopic examination, the pulmonary lesions included severe hyperaemia, haemorrhage, alveolar oedema, and alveolar emphysema. In the thymus, there was degeneration and necrosis of Hassall's corpuscles and centrilobular haemorrhages. Lesions in kidneys included severe generalized cloudy swellings and multiple foci of early coagulative necrosis of the proximal convoluted tubules, general hyperaemia of the glomerular tufts, and degeneration of the glomerular tuft cells.

In Marschang & Crainiceanu's (1971) study on the effects of ammonia in the air of calf stables, ammonia concentrations reportedly ranged from 0.7 to 140 mg/m^3 (1 - 200 ppm). During

these periods of high ammonia concentration, high mortality rates were observed among the calves. The authors suggested that the high ammonia content weakened the resistance of the animals and thus created conditions for the development of secondary infections. Deaths were mainly caused by respiratory diseases. Autopsy indicated various types of change in the lungs, chiefly inflammation.

Air-ammonia concentrations in 3 cattle-fattening facilities in Romania were measured by Marschang & Petre (1971), who found concentrations ranging from 2 to 1400 mg/m^3 (3 - 2000 ppm). Morbidity (mainly from respiratory disease) and mortality rates increased with ammonia concentrations in the stalls and decreased as some of the toxic gas levels decreased to admissible concentrations. The authors suggested that ammonia is the most important environmental factor in producing disease in cattle-fattening stalls. They did not refer to the growth rate of the cattle; however, in an additional report, Marschang (1972) observed a marked decrease in the growth rate of fattening cattle, when the ammonia content of the stable was high.

(b) *Monogastric animals*

Monogastric animals are considered relatively tolerant to dietary urea, since they lack the large amounts of bacterial urease present in the rumen of ruminants. Horses may frequently consume cattle rations that contain urea or other non-protein-nitrogen sources.

Hintz et al. (1970) found that the urease activity in the caecal fluid from ponies was 17 - 25% of that reported for bovine rumen fluid. They subjected 8 ponies to oral doses of urea at 3.3 - 3.6 g/kg body weight, to study the toxic effects of urea overdosage. Seven of the ponies died of ammonia toxicity, 3 - 12 h after treatment. Clinical signs of toxicosis were characteristic of severe central nervous system derangement. These signs were similar to those previously reported for ruminants, with the exception of head pressing against a fixed object prior to loss of coordination. No significant gross lesions were observed on necropsy. Blood-ammonia increased linearly until death. Blood-alpha-keto-glutarate decreased initially, reached minimal values at about 30 min and then increased to 3 times the zero time value. Blood-glucose remained constant for the first 2.5 h and then increased to about 3 times the initial value. Blood-pyruvate decreased during the first 3.5 h and then increased to 10 times the initial values.

6.5.2.2. Inhalation exposure

(a) *Swine*

The acute inhalation effects of ammonia in swine are given in Table 15.

(b) *Poultry*

As discussed previously, poultry are exposed to ammonia in the atmosphere of poultry houses; this ammonia is released from the action of bacteria on poultry wastes. The toxic effects of this exposure are primarily seen in the eyes and respiratory tract.

An idiopathic ocular disorder in young chicks, designated keratoconjunctivitis, was first described by Bullis et al. (1950), who attributed it to environmental factors in the rearing

facilities.

Anderson et al. (1964a) reported that chickens exposed continuously to ammonia at 14 mg/m^3 (20 ppm) showed some signs of discomfort, including rubbing of the eyes, slight lachrymation, anorexia, and, later, weight loss. Chickens exposed to ammonia at 14 mg/m^3 for as little as 72 h were more susceptible to aerosol infection with Newcastle disease virus. Gross and microscopic damage to the respiratory tract could be detected after 6 weeks of continuous exposure to ammonia at 14 mg/m^3. Valentine (1964) reported tracheitis in chicks exposed to ammonia at 42 - 49 mg/m^3 (60 - 70 ppm). The breathing of the birds was audible as moist rales with bubbling sounds. At post-mortem examination, some of the birds had slight congestion of the lungs with excess mucous in the respiratory tract. The mucous membranes of the trachea were much thicker than in the control birds, and there was leukocytic infiltration of the tissue. It was suggested that this tracheitis may predispose the affected birds to respiratory diseases with the added risks of secondary infections.

Charles & Payne (1966a) reported that exposure to atmospheric ammonia at 70 mg/m^3 (100 ppm) caused a reduction in carbon dioxide production and depth of respiration and a 7 - 24% decrease in the respiration rate of laying hens. The authors also observed that broilers reared to 28 days of age in atmospheres containing high concentrations of ammonia consumed less food and grew more slowly than unexposed chickens. Pullets reared in high-ammonia atmospheres matured up to 2 weeks later than pullets reared in ammonia-free atmospheres.

Airsacculitis, one of many respiratory diseases in poultry, has been associated with high ammonia concentrations in poultry houses (Ernst, 1968). High concentrations of dust were also noted during periods of winter confinement, when high ammonia concentrations were observed. The incidence and severity of air-sac lesions in turkeys increased signficantly with high concentrations of dust (0.6 - 1.0 mg/m^3 or 21 - 35 mg/m^3) in the atmosphere. Flocks with a high rate (47%) or a low rate (2%) of infection with *Mycoplasma meleagridis* were similarly affected. No significant interaction between dust and ammonia concentrations (up to 21 mg/m^3 or 30 ppm) with regard to effects on the development of air-sac lesions was found. Mortality rate and feed conversion were not significantly affected by exposure to dust and ammonia. There was considerable loss of cilia from the epithelium of the tracheal lumen and an increase in mucous-secreting goblet cells in turkeys exposed to high concentrations of dust and ammonia. Areas of consolidation and inflammation were frequently observed in the lungs of these turkeys. The air-sac lesions ranged from mild (lymphocytic infiltration) to severe (masses of gaseous material).

Airsacculitis has also been experimentally induced in chickens exposed to atmospheric ammonia and the stress of infectious bronchitis vaccination (Kling & Quarles, 1974). Eighty Leghorn male chicks were maintained in 12 controlled-environment chambers. Ammonia at 0, 17.5, or 35 mg/m^3 (0, 25, or 50 ppm) was introduced into the chambers from the 4th to 8th weeks of age. An infectious bronchitis vaccination was administered to all chicks at 5 weeks of age. Body weights and feed efficiencies were determined at 4, 6, and 8 weeks. At 4, 5, 6, and 8 weeks, lung and bursae of Fabricius weights, haematocrits, and air-sac scores were determined. Body weights and feed efficiencies were significantly reduced in the ammonia chambers. The bursae of Fabricius in the ammonia-stressed chickens were significantly larger than those of controls at 5

weeks of age and significantly smaller at 8 weeks of age. Chickens grown in ammoniated environments had significantly larger lungs at 8 weeks. Haematocrits were not significantly different among treatments. Total air-sac scores were significantly higher in the ammonia-stressed chickens at 8 weeks. The results indicated that chickens were stressed by the ammonia at 17.5 or 35 mg/m^3, and by the infective bronchitis vaccination. In a similar study (Quarles & Kling, 1974), exposure of broiler chicks to 17.5 or 35 mg/m^3 (25 or 50 ppm) from the 4th to the 6th week resulted in the observation of severe airsacculitis at 6 and 8 weeks of age. During the test, airborne bacterial counts were significantly higher in chambers with ammonia than in control chambers.

Table 15. Acute inhalation effects of ammonia in swine

Ammonia concentration	Dose	Effects	Refe
196 mg/m^3 (280 ppm)	single	frothing of the mouth and excessive secretion; after 36 h, convulsions occurred, and breathing was extremely short and irregular; the effects ceased after a few h	Stomb
7, 35, 70, or 105 mg/mg^3 (10, 50, 100, or 150 ppm)	5 weeks	high concentrations (70 and 105 mg/m^3) appeared to cause excessive nasal, lachrymal, and mouth secretion after 3 - 4 days of exposure at 35 mg/m^3, the secretory rate was only slightly higher than that in control animals; after 1 - 2 weeks of exposure, the signs noted appeared to lessen gradually; examination of respiratory tract did not reveal any significant gross- or microscopic differences related to ammonia exposure	Stomb
0, 35, 70, or 105 mg/m^3 (0, 50, 100, or 105 ppm)	4 weeks	decrease in pig growth was noted at all concentrations; at 35 or 70 mg/m^3, pigs converted feed to body weight gain more efficiently than either controls or pigs exposed to 105 mg/m^3; an acute imflammatory reaction in the tracheal epithelium and a mild-to-heavy exudate in the turbinate lumen were observed at 70 and 105 mg/m^3, only	Drumm
0 and 70 mg/m^3 (100 ppm) (1 - 7 weeks old) 0 + dust (100 ppm + dust)	2 - 6 weeks	conjunctival irritation after the first day which persisted for 1 week; dust alone had no effect; histopatho-logical changes were limited to the nasal and tracheal epithelium; there was no evidence of structural damage in the bronchial epithelium or alveoli	Doig

Charles & Payne (1966b) studied the effects of graded concentrations of atmospheric ammonia on the performance of laying hens. At 18 °C, ammonia at 73.5 mg/m^3 (105 ppm) significantly reduced egg production, after 10 weeks of exposure. No effects were observed on egg quality. Food intake was reduced and weight

gain was lower. No recovery in egg production occurred when the treated groups were maintained for an additional 12 weeks in an ammonia-free atmosphere. Similar results were observed at 28 °C, under the same conditions. Earlier work had indicated that egg quality could be affected by ammonia exposure (Cotterill & Nordskog, 1954). Freshly-laid eggs were exposed to various concentrations of ammonia in a desiccator for 14 h at room temperature and then moved to normal atmosphere for another 32 h at 50 °C, before examination. There was evidence of absorption of ammonia into the eggs and significant impairment of interior egg quality, as measured by Haugh units, pH, and transmission of light. The authors suggested that the quality of eggs left all day in hen houses containing high concentrations of ammonia might be adversely affected.

7. KINETICS AND METABOLISM

Ammonia, a by-product of protein and nucleic acid metabolism and a minor component of the diet, is in a state of flux in the body, though it is present in low steady-state concentrations in body fluids. In animals, metabolically-produced ammonia is conjugated and excreted. Toxicity will only occur if these conjugation and excretion mechanisms are defective, or if they are overwhelmed by excessive exposure.

7.1. Absorption

7.1.1. Respiratory tract

Egle (1973) studied the retention, over a short period of time, of inhaled ammonia in air at concentrations in the range 150 - 500 mg/m^3 (214 - 714 ppm), in mongrel dogs of both sexes (7 - 37 per study). Retention was not materially affected by respiratory rate, tidal volume, or concentration. Retention by the whole respiratory tract averaged 78%, but the complexity of the dynamics of this retention is illustrated by the fact that when respiration was via an endotracheal tube, limiting exposure to the lower respiratory tract, and when the upper respiratory tract (muzzle to tracheal bifurcation) was perfused tidally, the retention was 78% in each case. However, unidirectional perfusion of the upper respiratory tract with ammonia in air produced a higher mean retention of 89%.

Schaerdel et al. (1983) exposed 4 groups of rats, 8 per group, to average ammonia concentrations of 11, 23, 220, or 826 mg/m^3 (15, 32, 310, or 1157 ppm) for 24 h. Ammonia in blood was measured at 0, 8, 12, and 24 h. At the 2 lowest concentrations, there was no increase in blood-ammonia. However, after 8 h at 220 and 826 mg/m^3, significant increases of 0.192 and 0.244 mmol (3.26 and 4.18 mg/litre) were noted. After 12 and 24 h, the increases were not so marked, indicating an increase in ammonia metabolism.

In a study by Silverman et al. (1949), 7 male volunteers were exposed to 350 mg/m^3 (500 ppm) for 30 min. Initial ammonia retention was not reported for all subjects, but, in one instance, was around 75%. Retention decreased progressively until at equilibrium it was 23% (range 4 - 30%); equilibrium was reached in 10 - 27 min. Some irritation was noted in the nose and throat, leading to the suggestion that ammonia at this concentration was primarily absorbed by the upper respiratory tract. Levels of blood-urea-nitrogen (BUN), non-protein nitrogen, urinary-urea, and urinary-ammonia remained normal.

In another study (Kustov, 1967), exposure of human volunteers

to ammonia for a longer duration (14 mg/m^3 (20 ppm) for 8 h) was accompanied by a statistically-significant increase in BUN from 23.9 to 30 mg%.

An early study was conducted by Landahl & Herrmann (1950) on the retention of gases by the human nose and lung. At ammonia concentrations of between 40 and 350 mg/m^3 (57 and 500 ppm) and a mean minute volume of 6 - 7 litre/min, for short durations (< 2 min), they found that approximately 92% ± 2% was retained in the respiratory system (i.e., mouth, lungs, etc.) in 2 male volunteers, tested 4 times. Differences in concentrations of ammonia did not affect retention values. In a separate study, about 83% was retained in the nasopharnyx at a flow rate of 18 litre/min, but only 63 - 71% was retained when the flow rate was tripled. These data are consistent with, though somewhat higher than, those reported by Egle (1973) for exposure in dogs.

It should be noted that experimental animals kept in cages may be exposed to relatively high concentrations of ammonia, even exceeding 100 mg/m^3, due to the degradation of urea in urine and faeces (Flynn, 1968; Schaerdel et al., 1983).

Because ammonia is very water soluble, and thus absorbed by the mucous coating in the upper respiratory tract, the lungs are protected from the effects of exposure to low concentrations of ammonia (Haggard, 1924; Boyd et al., 1944). At the levels of ammonia associated with ambient air (i.e., 1 - 200 µg/m^3), very little, if any, is absorbed through the lungs.

If a person breathes an ammonia concentration in air of 18 mg/m^3 (a common occupational exposure limit) at 1 m^3/h and, if all the ammonia is retained, then 18/60 = 0.3 mg ammonia/min would require to be cleared by hepatic blood flow (say 1 litre/min). The rise in systemic blood-ammonia would be calculated at 0.3 mg/litre or 0.018 mmol. If the more realistic assumption of 30% retention were used, the corresponding increase in blood-ammonia concentration would be 0.09 mg/litre, about µmol. An arterial fasting ammonia concentration of 1.05 mg/litre has been reported in healthy subjects (Conn, 1972), so the calculated rise is only 10% over fasting levels.

7.1.2. Gastrointestinal tract

Ammonia is a trace compound in foods. Ammonia that is absorbed from the intestinal tract arises primarily from the bacterial degradation in the intestine of amino and nucleic acids from 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. The estimated ammonia production from various substrates in the human intestines ranges from 10 mg/day in the duodenum to 3080 mg/day in the colon and faecal contents. Nearly all the ammonia formed is absorbed (about 99% or 4000 mg). In healthy individuals, absorbed ammonia is mainly catabolized rapidly in the liver to urea; therefore, relatively small amounts reach the systemic circulation after absorption from the gastrointestinal tract as a consequence of this "first pass effect" (Summerskill & Wolpert, 1970).

Castell & Moore (1971) have shown that ammonia uptake from the human colon, the major site of ammonia production, increases with increased pH of the luminal contents. A similar effect of pH has been shown for the absorption of ammonia from the rumen of sheep (Hogan, 1961; Bloomfield et al., 1962). Since an increase in pH increases the proportion of non-ionized ammonia, the authors

concluded that simple non-ionic diffusion was responsible for the majority of ammonia transport. Evidence also exists for the active transport of the ammonium ion from the intestinal tract. Castell & Moore (1971) showed that ammonia transport by the human colon, though greatly diminished, still occurred when the luminal pH was reduced to 5, at which value non-ionized ammonia would be virtually absent. Mossberg & Ross (1967) and Mossberg (1967) studied the absorption of ammonia from isolated intestinal loops of the golden hamster and found that the ileal movement of ammonia against a concentration gradient was inhibited by cyanide, dinitrophenol, and anaerobiosis. This suggested that an energy-dependent transport system was operable in the ileum, where ammonia was absorbed preferentially, but not in the jejunum.

Membrane transport of the ammonium ion by the human erythrocyte has been demonstrated (Post & Jolly, 1957).

7.1.3. Skin and eye

Ammonia is highly mobile in all tissues and the ammonium ion readily penetrates the corneal epithelium. Within 5 seconds, traces are present in the anterior chamber of the eye (Siegrist, 1920). However, systemic and intra-ocular absorption by these routes are not quantitatively important.

7.2. Distribution

The ammonia normally present in all tissues in the body constitutes a dynamic pool throughout which absorbed ammonia is distributed. The distribution of total ammonia between body compartments is strongly influenced by pH. The non-ionized NH_3 is freely diffusible, whereas NH_4+ is less diffusible and relatively confined in compartments. The lower the pH of a compartment, the greater its total ammonia content (NRC, 1979).

The fate of absorbed ammonia molecules has been studied in man, by measurement of blood constituents, and, in experimental animals, by following the distribution of ^{15}N after the administration of $^{15}NH_3$ and compounds.

7.2.1. Human studies

In human beings, inhalation of ammonia (350 mg/m^3; 500 ppm) for 30 min did not have any effect on blood-nitrogen levels (Silverman et al., 1949). In another study, exposure of human subjects to 14 mg ammonia/m^3 (20 ppm), for a duration of 8 h, revealed a statistically-significant increase in BUN (from 23.9 to 30 mg%) (Kustov, 1967). However, this is unlikely to have represented the metabolic conversion of absorbed ammonia, since the increase was far greater than could have been accounted for by the quantity of ammonia inhaled.

Administration of 9 mg NH_4Cl/kg body weight, orally, to 20 healthy adult male and female volunteers caused a transient increase in ammonia concentrations in arterial blood in approximately half of the subjects. Concentrations peaked (mean, 1.4 mg NH_3/litre) at 15 min and returned to fasting levels (mean, 1.05 mg NH_3/litre) by 30 min. However, in 50 male patients with cirrhosis of the liver, blood-ammonia levels increased from already elevated fasting levels (mean, 1.56 mg NH_3/litre) to much higher peak concentrations (mean, 3.7 mg NH_3/litre) at 15 min, followed by a slow decrease reflecting impaired hepatic urea synthesis. Blood-

ammonia levels, before and after administration of ammonium chloride, were significantly higher among cirrhotic patients with portacaval anastomoses than among patients lacking such shunts (Conn, 1972).

7.2.2. Animal studies

The distribution, as well as the metabolic fate of ammonia, depends on the route of administration. After intestinal absorption, ammonium ions are primarily transformed by the liver to urea, and subsequently excreted in the urine. In contrast, intravenously-administered ammonium salts are more available as non-essential nitrogen for protein synthesis (Furst et al., 1969). However, some orally-administered ammonia, has been found to be incorporated into tissue proteins. Incorporation of ^{15}N was higher in serum globulins than in albumin after intravenous dosing with ^{15}N-ammonium salts, but this order was reversed after oral administration (Furst et al., 1970). The amount incorporated into protein by this route was greater, when protein intake was restricted (Richards et al., 1968).

Duda & Handler (1958) analysed the tissue of rats, 15 min after an intravenous injection of ^{15}N-ammonium lactate, and found that its major metabolites, glutamine, and urea, were quickly distributed throughout the body. The highest levels of labelled urea (in µmoles ^{15}N/g tissue) were found in the kidney (0.0217) and liver (0.0159), while lesser amounts were found in the heart (0.0086), spleen (0.0067), brain (0.0029), testes (0.0027), and carcass (0.0070). The highest levels of labelled glutamine (µmoles ^{15}N/g tissue) were found in the heart (0.086) and liver (0.055) and lesser amounts (0.005 to 0.032) in the brain, spleen, carcass, kidney, and testes.

Vitti et al. (1964) examined the distribution of ^{15}N from ammonium citrate, administered by different routes, into the proteins of various tissues of hypophysectomized rats. The liver, kidney, and spleen contained greater concentrations of ^{15}N incorporated into proteins than heart or muscle fractions during 72 h following intragastric, intraperitoneal, and subcutaneous administration of ^{15}N-ammonium citrate. After the first 6 h, during which the intragastric route gave higher values, the quantity of ^{15}N incorporated into liver-protein was not substantially affected by the route of administration. In most of the other tissues studied, however, ^{15}N incorporation tended to be least by the intragastric route, followed, in increasing order, by the intraperitoneal and subcutaneous routes. By the last route, more labelled ammonia was apparently made available to the widely distributed glutamine-synthetase (EC 6.3.1.2) system (section 7.4.3).

7.3. Metabolic Transformation

Most organisms have mechanisms for conjugating ammonia into non-toxic compounds for excretion. Terrestrial mammals synthesize urea, which requires the concerted action of several enzymes of the Krebs-Henseleit (urea) cycle. One of these enzymes, glutamine synthetase (EC 6.3.1.2), was present in the brains of all vertebrate species examined. Glutamine synthetase was also present at significant levels in the liver in all organisms examined (Brown et al., 1957).

Exogenous ammonia, administered intravenously as an ammonium

compound, is metabolized to glutamine as the major early product (Duda & Handler, 1958). The ammonia fixed in glutamine may eventually end up in amino acids, purines, pyrimidines, or other nitrogen-containing compounds. Ingested ammonium chloride or endogenous ammonia is absorbed into the portal vein and converted in the liver to urea (Furst et al., 1969; Goodman & Gilman, 1970; Pitts, 1971).

Results of studies on the metabolic fate of dietary ammonium citrate (Foster et al., 1939) and intravenously-administered ammonium lactate (Duda & Handler, 1958) in rats showed that urea synthesis represented a nearly constant fraction of the administered ammonia over a large concentration range. Besides glutamine and urea, labelled nitrogen also appeared in creatine, glycine, alanine, proline, histidine, arginine, glutamic acid, and aspartic acid. Vitti et al. (1964) examined the incorporation of ^{15}N from ammonium citrate into proteins of liver, heart, kidney, spleen, and muscle fractions of untreated and growth hormone-treated, hypophysectomized rats, and found differences in the metabolic fate, depending on the route of administration. Subcutaneous injection facilitated the labelling of amide nitrogen, indicating extensive disposition via glutamine synthesis. In contrast, intragastric or intraperitoneal administration resulted in the labelling of arginine, glutamic acid, and other alpha-amino acids of the liver. Amide-nitrogen was labelled to a much lesser extent than by the subcutaneous route. The tissue distribution of the label also differed according to the route of entry (section 7.2.2).

7.4. Reaction with Body Components

Ammonia-nitrogen is central in nitrogen metabolism and therefore becomes incorporated in all proteins and nitrogen-containing components in the course of metabolic turnover. Ammonia does not react with body components in the manner of alkylating agents or compounds that modify haemoglobin.

7.5. Elimination and Excretion

7.5.1. Expired air

Ammonia may be excreted through expired air. Hunt (1977) reported human expired air levels of ammonia of between 105 and 2219 $\mu g/m^3$; Larson (1977) reported values of between 196 and 1162 $\mu g/m^3$, during mouth breathing. These values are higher than those expected from equilibration with plasma- and lung-parenchyma-ammonia levels (28 - 49 $\mu g/m^3$). This is most likely due to the synthesis of ammonia from salivary urea by oral microflora (Biswas & Kleinberg, 1971). Measurable amounts of free ammonia were also found in air expired by dogs given ammonium acetate intravenously (Robin et al., 1959), and normal dogs and human beings with hepatic-induced ammonia toxicity (Jacquez et al., 1957, 1959). Bloomfield et al. (1962) reported the presence of free ammonia in expired air from sheep during experimentally-induced urea toxicity. Normal levels of ammonia in the expired air of the rat have been reported to range from 7 to 247.1 $\mu g/m^3$, with a mean of 54.6 $\mu g/m^3$ in nose-breathing animals and 23.8 - 520.8 $\mu g/m^3$, with a mean of 200.2 $\mu g/m^3$ in tracheal-cannulated animals (Barrow & Steinhagen, 1980). The presence of ammonia in the expired air of human beings and experimental animals suggests that reaction products may be formed with a variety of airborne chemicals, thereby altering their toxicity.

7.5.2. Urine and faeces

Free ammonia is excreted by ammonotelic organisms (e.g., fish), uric acid by uricotelic animals (e.g., birds), and urea by ureotelic animals (e.g., mammals). Mammals may also secrete ammonia directly into the urine. Glutaminase (EC 3.5.1.2) catalyses the release of ammonia in the kidney tubular epithelium, where it serves as an acceptor of H^+ and regulates the acid-base balance (Van Slyke et al., 1943; White et al., 1973). In acidosis, the renal concentration of glutaminase increases over several days, paralleling the increased excretion of ammonium ions (Davies & Yudkin, 1952; Muntwyler et al., 1956; Kamin & Handler, 1957); two-thirds of the urinary-ammonia is contributed by this pathway (Van Slyke et al., 1943), and approximately one-third by protein metabolism and ammonia clearance from the plasma by the kidney.

Oral and intravenous administration of ammonium lactate to healthy human volunteers produced different patterns of excretion, reflecting the effective barrier of the liver in preventing ingested ammonia from gaining access to peripheral circulation by converting most of the ammonia load to urea. Urinary-ammonia excretion was increased 8-fold and urea excretion was reduced by one-half after intravenous injection, as opposed to oral administration (Gay et al., 1969), probably due to the anabolism involved in the "first pass" effect after oral administration.

Less than 1% of the 4 g total ammonia produced in the human intestinal tract, per day, is excreted in the faeces (Summerskill & Wolpert, 1970).

7.6. Retention and Turnover

Some nitrogen derived from absorbed ammonia is incorporated in amino acids and proteins. The rate of ammonia-derived nitrogen turnover is rapid, but depends on the nutritional state. Thus, when $^{15}NH_4Cl$ had been administered orally to healthy male volunteers, for one week, 70% ^{15}N was excreted by those on a 70-g protein/day diet, while only about 35% ^{15}N was excreted by those on 20 g protein/day (Richards et al., 1968, 1975).

7.7. Uptake and Metabolism in Plants

Ammonia is used by many plants and preferentially by a few. However, ammonia is toxic, and its uptake in large quantities may put a severe strain on the carbohydrate metabolism of the plant in the provision of carbon skeletons for its detoxification. The absorption of ammonium usually is coupled with the exchange of cations as H^+. Ammonia-nitrogen functions as a nitrogen source for the synthesis of amino acids, which are incorporated in proteins. Plants that are able to absorb it in large amounts include many acid plants, such as Rumex, which are able to detoxify ammonia by forming ammonium salts of organic acids. "Amide plants", such as beet, spinach, and squash, are able to form large amounts of the amides, glutamine, and asparagine and can withstand quite high concentrations of ammonium salts by detoxifying the ammonia. Certain plants, such as rice, which live in water-logged anaerobic soils, require NH_3 or reduced organic nitrogen fertilizer, alone.

8. EFFECTS ON EXPERIMENTAL ANIMALS AND *IN VITRO* TEST SYSTEMS

8.1. Single Exposures

8.1.1. Inhalation exposure

LC_{50} studies and studies to determine the threshold for irritating effects on the respiratory system for the rat and mouse are summarized in Tables 16 and 17, respectively.

Table 16. Lethal concentrations (1-h exposure) of ammonia for rats and mice[a]

Measured concentration (mg/m^3)	Species	Mortality ratio[b]	Mean weight gain of survivors at 14 days (g)
4347	rat	0/10	3.5
5474	rat	8/10	-[c]
6888	rat	9/10	-
controls	rat	-	21.4
2520	mouse	0/10	-0.2
3185	mouse	3/10	-0.7
4004	mouse	9/10	-
controls	mouse	-	1.6

[a] Adapted from: MacEwen & Vernot (1972).
[b] Number dead/number exposed.
[c] Not enough survivors for comparison.

The acute lethal dose of ammonia by inhalation has been determined for both the rat and the mouse (MacEwen & Vernot, 1972). The results are summarized in Table 16. Male CFE rats ranging in weight from 200 to 300 g and male CF1 mice weighing from 20 to 30 g (ICR derived) were exposed for 1 h to several concentrations of ammonia. Inhalation of ammonia gas produced immediate nasal and eye irritation followed by laboured breathing and gasping in all test groups. In addition, convulsions were seen in mice. Surviving rats necropsied after 14 days showed moderate mottling of the liver, regarded as probable fatty infiltration, at the 5474 and 6888 mg/m^3 (7820 and 9840 ppm) dose levels. Mice surviving the 2 highest dose levels of 3185 and 4004 mg/m^3 (4550 and 5720 ppm) showed mild congestion of the liver. Pathological lesions were not seen in rats exposed to 4347 mg/m^3 (6210 ppm) or mice exposed to 2520 mg/m^3 (3600 ppm). The calculated 1-h LC_{50} values for the rat and the mouse were 5137 and 3386 mg/m^3 (7338 and 4837 ppm), respectively.

In another inhalation study, an LC_{50} value for the rat, with a 2-h exposure, was 7600 mg/m^3 (10 860 ppm) (Alpatov, 1964). In a further study, the threshold for acute effects (depression, then hyperactivity and convulsions) for a 2-h exposure was 85 mg/m^3 (121 ppm) (Alpatov & Mikhailov, 1963).

Table 17. Single-dose inhalation studies (LC_{50})

Species	Exposure time (min)	LC_{50} (mg/m^3)	Reference
rat	120	7600	Alpatov (1964)
rat	60	5137	MacEwen & Vernot (1972)
rat	5	18 693	Prokop'eva et al. (1973)

rat	15	12 160	Prokop'eva et al. (1973)
rat	30	7035	Prokop'eva et al. (1973)
rat	60	7939	Prokop'eva et al. (1973)
rat	10	31 612	Appelman et al. (1982)
rat	60	11 620	Appelman et al. (1982)
mouse	10	7060	Silver & McGrath (1948)
mouse	60	3386	MacEwen & Vernot (1972)
mouse	60	2960	Kapeghian et al. (1982)

Kapeghian et al. (1982) reported an acute inhalation toxicity study on male ICR mice, in which the 1-h LC_{50} with a 14-day observation period was calculated to be 2960 mg/m^3 (4230 ppm). Lungs of mice that died during exposure 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. Focal atelectasis was evident in survivors sacrificed after the observation period. Liver damage was also seen in these mice. There was evidence of swelling and increased cytoplasmic granularity of hepatocytes at 2408 mg/m^3 (3440 ppm) and scattered foci of frank cellular necrosis at 2954 mg/m^3 (4220 ppm). At 3402 mg/m^3 (4860 ppm), necrosis was increased. The liver lesions may have resulted from the compromised nutritional state of the mice. Follicular hyperplasia in the spleen was also seen in surviving animals, but this was absent in animals that died during exposure.

The acute LC_{50} in male and female Wistar rats was 31 612 mg/m^3 (40 300 ppm) for a 10-min exposure, 20 017 mg/m^3 (28 595 ppm) for a 20-min exposure, 14 210 mg/m^3 (20 300 ppm) for a 40-min exposure, and 11 620 mg/m^3 (16 600 ppm) for a 60-min exposure (Appelman et al., 1982). Survivors were observed for 14 days. Clinical signs of restlessness, eye irritation, nasal discharge, mouth breathing, and laboured respiration were seen during exposure. Gross necropsy revealed haemorrhagic lungs in animals that died during the study as well as in survivors. No histopathology was performed.

Prokop'eva et al. (1973) reported that white rats exposed to high concentrations of ammonia (6000, 3000, 1000 mg/m^3 or 6814, 4307, 1436 ppm) for periods of 5, 15, 30, and 60 min exhibited dyspnoea, irritation of the respiratory tract and eyes, cyanosis of the extremities, and increased excitability. The LC_{50} values for inhalation exposures of 5 and 15 min were 18 693 mg/m^3 (26 704 ppm) and 12 160 mg/m^3 (17 372 ppm), respectively, while for 30 and 60 min, the values were 7035 mg/m^3 (10 050 ppm) and 7939 mg/m^3 (11 342 ppm), respectively. Inhalation of ammonia at concentrations of 3000, 1000, or 300 mg/m^3 (4307, 1436, 431 ppm) resulted in a drop in static muscular tension, leukocytosis, prolongation of the latent reflex time, increase in total protein and blood sugar, increased oxygen consumption, and a rise in the level of residual nitrogen. No changes were observed in rats exposed to a concentration of ammonia of 100 mg/m^3 (144 ppm), for 5, 15, 30, and 60 min. Animals exposed to high concentrations of ammonia (exact concentration not specified) developed pneumonia (Prokop'eva et

al., 1973).

When exposed to toxic levels of ammonia, within 1 min, mice exhibited excitement, closing their eyes immediately and gasping (Silver & McGrath, 1948). Groups of 20 mice were exposed to ammonia gas for 10 min at 9 concentrations ranging from 6100 to 9000 mg/m^3 (8758 to 12 921 ppm). The median lethal concentration, calculated from the mortality at 10 days, was 7060 ± 320 mg/m^3 (10 152 ± 460 ppm). Most (93%) deaths occurred rapidly, due to convulsions after 5 min of exposure. Survivors usually recovered within 10 min.

The LC_{50} values for the rat and the mouse are summarized in Table 17.

Dose-dependent ultrastructural changes in the terminal airways of mice exposed for 3 - 60 min to ammonia (concentration unspecified) included oedema of the alveolar epithelium, development of intracapillary platelet thrombosis, increased secretions by the Clara cells, presumed to be phospholipids, and an increase in the number of empty lamellar bodies in the large alveolar cells (Niden, 1968).

Twenty cats were anaesthetized and exposed to 700 mg/m^3 (1000 ppm) for 10 min via endotracheal tube and then observed for up to 35 days (Dodd & Gross, 1980). All cats had severe dyspnoea, anorexia, and dehydration, 24 h after exposure. Several measures of pulmonary function were impaired. Gross pathology of the lungs showed various degrees of congestion, haemorrhage, oedema, interstitial emphysema, and collapse, all non-specific for any post-exposure day. Bronchopneumonia was common, 7 days after exposure.

Barrow et al. (1978) exposed male Swiss-Webster mice to concentrations of ammonia ranging from 70 to 560 mg/m^3 (100 - 1000 ppm) for 30 min. The average respiratory rate depression in 4 mice for each of 4 exposure levels was evaluated. The maximum depression in respiratory rate at each exposure level occurred within the first 2 min. The concentration expected to elicit a 50% decrease in respiratory rate (RD_{50}) in mice, calculated by a regression equation for ammonia, was 212 mg/m^3 (303 ppm) with 95% confidence limits of 132 - 343 mg/m^3 (188 - 490 ppm). Effects of ammonia exposure such as bradycardia and peripheral vasoconstriction accompanied respiratory rate depression at the RD_{50} and above.

A concentration of 350 mg/m^3 (500 ppm) was reported by Wood (1979) to be the level at which unrestrained mice did not consistently adopt avoidance measures on inhalation of ammonia. Both this author and Barrow et al. (1978) claimed to offer more sensitive end-points for assessing ammonia irritation. However, the no-observed-adverse-effect level reported by Wood (1979) was higher than the concentration determined by Barrow et al. (1978) to elicit 50% depression of respiratory rates in mice.

Dalhamn & Sjoholm (1963) tested *in vitro* preparations of the tracheas of 8 rabbits. Arrested ciliary activity was observed after 5 min of exposure to ammonia at 350 - 700 mg/m^3 (500 - 1000 ppm). In *in vivo* studies on rabbits, Dalhamn (1963) showed that the level of ammonia entering the nasal cavity, necessary to cause small changes in the rate of tracheal ciliary beating was 1400 mg/m^3 (2000 ppm). This corresponds to a tracheal

concentration of approximately 70 mg/m^3 (100 ppm).

When anaesthetized male rabbits were exposed to 8 ammonia concentrations ranging from 700 to 14 000 mg/m^3 (1000 - 20 000 ppm) (Richard et al., 1978b), bradycardia appeared at 1750 mg/m^3 (2500 ppm). Hypertension, cardiac arrhythmia, macroscopic lung changes, and EEG abnormalities were also reported. Signs appeared more rapidly at higher concentrations with the complete syndrome appearing at 3500 mg/m^3 (5000 ppm).

Mayan & Merilan (1972) exposed 16 adult female New Zealand white rabbits to ammonia concentrations of 35 and 75 mg/m^3 (50 and 100 ppm) for 2.5 h. The average decreases in respiratory rate, which were 34 and 32.7%, respectively, were significantly ($P <$ 0.01) less than control values. There were no histopathological changes in lung, liver, spleen, or kidneys.

Enzymatic alterations in rats following inhalation of low levels of ammonia have also been reported. At concentrations of approximately 20 - 121 mg/m^3 (29 - 173 ppm), there was a decrease in the activities of liver succinic dehydrogenase (EC 1.3.99.1), lactate dehydrogenase (EC 1.1.1.28), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and adenosine triphosphatase (EC 3.6.1.3). Liver acid phosphatase (EC 3.1.3.2) activity was increased (Zlateva et al., 1974).

When 180 mice were exposed for 10 min to ammonia at 6139 - 9058 mg/m^3 (8770 - 12 940 ppm), death with convulsions began to occur 5 min after exposure. One hundred mice died during the exposure. The surviving animals (80) recovered rapidly; however, 7 died between the sixth and tenth days following exposure (NRC, 1979).

8.1.2. Oral exposure

The effects of ammonia and its compounds are mainly of 2 types. The first is the effect of ammonia itself. The second is the effect of the anion bound to the ammonium ion. Ammonium chloride, especially, will mainly exert its effects in the mammalian body due to the formation of hydrogen chloride. Most of the experimental work concerning the oral route of administration has centred on ammonium chloride, which has been used extensively in the study of metabolic acidosis. There have been few studies in which attempts have been made to identify the role of the ammonium ion and ammonia in the effects (Table 18).

Median lethal doses (LD_{50}s) of 4400 and 3100 mg/kg body weight have been reported for ammonium sulfamate in the rat and the mouse, respectively. (Vinokurova & Mal'kova, 1963). A similar figure for the oral LD_{50} in the rat of 4520 (4070 - 5020) mg/kg was reported by Bukhovskaya et al. (1966). Frank (1948) reported a lethal dose for ammonium sulfate of between 3000 and 4000 mg/kg body weight in the rat.

8.1.2.1. Effects of metabolic acidosis induced by ammonium chloride

The ingestion of ammonium chloride in doses of around 500 - 1000 mg/kg body weight per day, for periods ranging from 1 to 8 days, has induced metabolic acidosis in mice, guinea-pigs, rats, rabbits, and dogs. However, Boyd & Seymour (1946) did not report any toxic effects at doses of up to 1 g/kg body weight in rats, rabbits, guinea-pigs, and cats (50 animals per group).

Clinical signs, depending on the severity of the acidosis, include: a decrease in plasma- and urinary-pH; decreased appetite; decreased carbon dioxide-combining power; an increase in BUN and chlorides; an increase in plasma proteins; an increase in haematocrit (haemoconcentration); increased gluconeogenesis; increased phosphoenolpyruvate carboxykinase (EC 4.1.1.49) activity; increased urinary ammonium; increased urea, sodium, chloride, calcium, and titratable acid excretion; an increase in malate and oxaloacetate concentrations in renal tissue; and decreased concentrations of glutamine, glutamate, and alpha-ketoglutarate in the kidney. Pulmonary oedema, central nervous system dysfunction, and renal changes are reported to have occurred after ingestion of ammonium chloride.

Susceptibility to ammonium chloride differs among species. For instance, pulmonary oedema is produced in cats, but not in rabbits; yet cats have been shown to be more resistant to oral poisoning by ammonium chloride than other animals studied.

Age is an important factor in the response of rats to oral doses of ammonium salts. Benyajati & Goldstein (1975) found that the administration of a single dose of 5 mmol ammonium chloride/kg body weight (267.5 mg/kg) by gavage to 7- to 12-day-old rats (39 animals) increased ammonia excretion by about 64%, within 4 h, compared to an increase of 155% in 20 adult rats after similar treatment.

Table 18. Selected studies on the acute oral effects of ammonia compounds in ra

Ammonium compound	Mode of administration	Sex, strain, age, or weight	Number	Dose	Effect
Urea	drinking-water	female, Holtzman, 200 - 250 g	6	concentration of N equivalent to that of 0.28 mol/litre NH_4Cl for 7 days *ad lib*	no renal hypertroph
Citrate	drinking-water	female, Holtzman, 200 - 250 g	6	0.28 mol/litre solution for 7 days *ad lib*	no renal hypertroph
Chloride	drinking-water	female, Holtzman, 200 - 250 g	9	0.28 mol/litre for 7 days, *ad lib* (about 1000 mg/kg per day)	metabolic acidosis; in kidney weight a N; increased capac produce ammonia fro glutamine in kidney hypertrophy; unilat nephrectomy plus ac induced by NH_4Cl ca greater hypertrophy either alone

Table 18. (contd.)

Ammonium compound	Mode of administration	Sex, strain, age, or weight	Number	Dose	Effect
Chloride	diet	female, 150 g	125	all on low-	consumption of food

protein diet; Group I: no NH_4; Group II: 1% NH_4Cl (300 mg/kg body weight per day); Group III: 2% NH_4Cl (380 mg/kg body weight per day); Group IV: 4% NH_4Cl (560 mg/kg body weight per day); Group V: 8% NH_4Cl (540 mg/kg body weight per day, for 7 days	related to dosage o dosage-related decr weight gain; increas value for ip dose of NH_4Cl in Groups II a indicating an incre adaptive capacity f detoxification; LD_{50} Group IV same as Gr LD_{50} value of Group than Group I; dose- decrease in blood-gl levels; decrease of and liver glycogen; dependent increase ornithine transamina ornithine transcarb increase in adenosi triphosphate concen blood	

8.1.2.2. Organ effects following oral administration

(a) *Lung and central nervous system*

Toxic doses of ammonium salts induced acute pulmonary oedema in rats, guinea-pigs, and cats, but not in rabbits given ammonium chloride (6% aqueous solution) intraperitoneally or by gavage, though the doses were sufficient to cause death. To induce similar effects in cats, a larger dose (unspecified) of ammonium chloride and a longer latency period (from 1 to 3 h) were required, indicating a greater resistance of this species to ammonium chloride (Koenig & Koenig, 1949).

In a limited study to assess the role of the ammonium ion in producing pulmonary oedema, Koenig & Koenig (1949) administered, by gavage, to 5 different guinea-pigs, 6 ml of 20% ammonium nitrate; 7 ml of 6.4% ammonium acetate; 7 ml of 10% ammonium bromide; 7 ml of 6% ammonium chloride; or 7 ml of 7.4% ammonium sulfate, respectively. The last 4 solutions contained approximately equivalent concentrations of ammonium. All 5 animals died of acute pulmonary oedema; the lungs of a control animal that had received water by gavage remained normal. The pulmonary oedema could not be attributed to the induced acidosis, since guinea-pigs and cats, made severely acidotic by gavage with sodium lactate or dilute hydrochloric acid, did not show lung oedema (Koenig & Koenig, 1949).

Progressive signs of ammonium poisoning caused by ammonium salts, given by gavage, indicative of both pulmonary and nervous system dysfunction, were reported in less than 30 min in guinea-pigs and rats (Koenig & Koenig, 1949) including:

(i) a rapid increase in the rate and depth of respiration;

(ii) weakness and difficulty in locomotion;

(iii) hyperexcitability for tactile, auditory, and painful stimuli; and

(iv) muscle fasciculations over most of the body followed

by generalized tonic convulsions and then coma.

The respiratory rate was greatly reduced but the depth of respiration increased and was accompanied by gasping and stridor. Histological changes occurred in both the lung and brain, while oedema, congestion, and haemorrhage were found principally in the lung (Koenig & Koenig, 1949; Cameron & Shiekh, 1951).

The addition of ammonium chloride to rat feed resulted in reduced dietary consumption (Motyl & Debski, 1977). The results of studies by Noda & Chikamori (1976) pointed to a direct effect of the ammonium ion on the brain area that regulates feeding. Rats with bilateral lesions in the prepyriform cortical area of the brain consumed as much diet containing 3% ammonium chloride as basal diet. A unilateral injection of 10 mg/litre of 2% NH_4Cl/kg

body weight into prepyriform cortical areas, in contrast to an injection into other areas of the brain, or an injection of sodium chloride, significantly reduced the food intake of 6 adult male Wistar rats. The results of these studies suggest that ammonium ions directly influence appetite by their effects on prepyriform cortical areas.

(b) *Kidney*

Lotspeich (1965) reported that ingestion for 7 days of 0.28 mol/litre (1.5%) ammonium chloride in water *(ad lib)* produced renal hypertrophy with new cell formation and an enlargement of existing cells. Some animals from each of the acidotic and control groups were subjected to unilateral nephrectomy. Highly significant increases in kidney wet weight, dry weight, and total nitrogen were observed in the acidotic group. Similar changes were produced by unilateral nephrectomy. In unilaterally nephrectomized rats made acidotic by the ingestion of ammonium chloride, the remaining kidney was larger than that seen in animals with unilateral nephrectomy without induced acidosis, suggesting an additive effect of unilateral nephrectomy and ammonium chloride intake.

The relationship between the renal hypertrophy and the ammonium ion, acidosis, non-specific intake of nitrogen, or increased solute load was examined (Lotspeich, 1965). Isomolar (0.28 mol/litre) solutions of sodium chloride and ammonium chloride produced renal hypertrophy. The increase due to sodium chloride was less marked (it was noted that the sodium chloride group drank 3 or 4 times as much solution each day as the ammonium chloride group). Isomolar (0.2 mol/litre) solutions of sodium bicarbonate and ammonium citrate did not induce renal hypertrophy. When 3 groups of 6 rats were given drinking-water containing 0.28 mol/litre sodium chloride, 0.28 ammonium chloride, or a urea solution containing nitrogen equivalent to that in the 0.28 mol/litre ammonium chloride, for 7 days, only the ammonium chloride solution induced acidosis. The rats in both the sodium chloride and ammonium chloride groups had larger kidneys than the urea-drinking rats, though the kidneys of the sodium chloride-drinking rats were not as large as those of the ammonium chloride-drinking rats. Since renal hypertrophy was produced by solutions containing the chloride ion, while ammonium citrate and urea did not cause any hypertrophy, the evidence does not support the hypothesis that renal hypertrophy is due to the ammonium ion.

Thompson & Halliburton (1966) also reported renal hypertrophy in rats that ingested 3% ammonium chloride in the diet for 6 days. The ingestion of ammonium citrate or sodium chloride in amounts

that were equivalent to that of the ammonium chloride did not cause renal hypertrophy.

Janicki (1970) examined the kidneys of rabbits that had been administered 16.2 g ammonium chloride, by gavage, over a period of 2 days and found the epithelium of the convoluted tubules swollen, vacuolated, and completely filling the lumen; nuclei exhibited karyolysis. Findings were similar in 4 rabbits administered 5 - 7 g ammonium chloride, by gavage, over a period of 3 - 7 days.

However, hyperaemia of the renal cortex of rabbits was noted after the administration, by gavage, of single doses of 0.8 and 1 g ammonium carbonate/kg body weight (Yoshida et al., 1957). As the carbonate ion does not have an acidifying effect, this suggests that the ammonium ion produces some effects on the kidney.

8.1.2.3. Influence of diet on the effects of ammonia

In 6 mongrel dogs, blood-urea levels were increased by a higher level of protein intake (6 g protein/kg per day) (Bressani & Braham, 1977). Levels of ~30 mg BUN/litre were found 8 h after the daily feed. When the protein intake was low (4 mg protein/kg per day), BUN levels of 14 mg/litre were reported 8 h after the daily feed. The frequency of protein intake did not affect the maximum value of blood-urea levels when the protein intake was low (4 mg protein/kg per day). This suggests that high levels of urea and ammonia in the blood might occur in animals that suffer from liver insufficiency and are fed high-protein diets.

Kulasek et al. (1975) studied the effects of nitrogen in the diet on ammonia detoxification, using 210 rats and 6 diets containing 50, 150, and 700 g casein/kg (low-, optimal-, or high-protein diet, respectively), with or without the addition of ammonium chloride at 20 g/kg. After 8 or 9 days of feeding, the LD_{50} from an intraperitoneal injection of a 2.7% solution of ammonium chloride was determined. The LD_{50} increased in proportion to the amount of nitrogen in the diet. The data suggest that higher doses of exogenous ammonia were tolerated by rats on protein-rich diets or diets containing an ammonium salt as an additive. An adaptive capacity for ammonia detoxification was further demonstrated by Motyl & Debski (1977) in a study using rats fed low-protein diets with the addition of NH_4Cl. The LD_{50} values for the ip injection of 2.7% NH_4Cl were higher in animals fed low-protein diets supplemented with NH_4Cl (Table 18). Stevens et al. (1975) raised weanling rats for 3 - 6 weeks on a protein-deficient diet. Subsequent challenge by a large, intraperitoneally-injected dose of ammonium chloride indicated that severe protein deprivation increased vulnerability to ammonia poisoning compared with that of control groups not prefed protein-deficient diets. Thus, with the exception of animals suffering from liver dysfunction, animals on high-protein diets seem able to tolerate a higher oral intake of an ammonium salt.

A group of eight, 6- to 8-month-old cats, given a single meal of a complete amino acid diet without arginine, developed hyperammonaemia with elevated plasma levels of glucose and ammonia and showed clinical signs of ammonia toxicity within 2 h. One cat died 4.5 h after ingesting only 8 g of the diet. Five of the surviving cats given a single meal of complete amino acids, in which arginine was replaced with an equivalent amount of ornithine, did not show any unusual signs. This finding indicates that the cat is unable to synthesize ornithine at the rate required by the

urea cycle to dispose of ammonia from amino acid catabolism; however, the dietary requirements for cats may differ from those of other adult animals, including human beings, because the cat has a much higher protein requirement, i.e., it requires approximately 20% of dietary calories as protein, as opposed to only 4 - 8% required by the rat, dog, sheep, and man (Morris & Rogers, 1978).

The lethal effects of ammonia poisoning have been prevented by the amino acids, ornithine and aspartic acid. To test whether this was mediated by a sparing action of adenosine triphosphate (ATP) on ammonia metabolism, 20 dogs with chronic Eck's fistula were injected in the duodenum with ammonium acetate at 4.1 mmol/kg body weight (~219.4 mg/kg). Half of the group was given 2 mg ATP/kg, 1 h prior to administration of the ammonium acetate. In 9 of the 10 dogs, ATP prevented a rise in levels of ammonia in venous blood. Grossi et al. (1968) administered ATP (2 mg/kg) intravenously to 6 dogs with chronic Eck's fistula and on high-protein diets. Within 1 h, high blood-ammonia levels returned to normal. In chronic liver disease, there may be insufficient ATP available for ammonia detoxification, resulting in hyperammonaemia.

8.1.3. Dermal exposure

No data are available regarding any systemic effects of dermal exposure to ammonia or ammonium compounds.

8.1.4. Effects due to parenteral routes of exposure

The parenteral toxicity of ammonia and ammonium compounds has been studied extensively. Toxicity is influenced by the route of administration, e.g., with oral administration, there is the capacity for detoxification of exogenous ammonia by the liver.

8.1.4.1. Lethality

The intravenous (iv) and intraperitoneal (ip) LD_{50} values for a number of ammonium compounds in various species are summarized in Table 19. The toxic syndrome was similar in all species studied. The signs, after iv injection, were characterized by immediate hyperventilation and clonic convulsions followed by either fatal tonic extensor convulsion or the onset of coma in 3 - 5 min. The animals remained comatose for approximately 30 - 45 min. At this stage, tonic convulsions and death can occur at any time, but animals that survive usually recover rapidly and completely (Warren, 1958; Warren & Schenker, 1964; Wilson et al., 1968a,b). After intraperitoneal injection, the signs did not appear until 15 - 20 min after administration (Greenstein et al., 1956; Wilson et al., 1968b).

Table 19. Toxicity of several ammonium compounds in selected species

Ammonium compound	Species	Intravenous dose (mmol/kg of body weight) (LD_{50})	Intraperitoneal dose (mmol/kg of body weight (LD_{50})
Acetate	rat	-	8.20
	mouse	6.23	-
	mouse	5.64	10.84
	chick	2.27	10.44
Bicarbonate	mouse	5.05	-
	mouse	3.10	-

Carbamate	mouse	0.99	-
Carbonate	mouse	4.47	-
	mouse	1.02	-
Chloride	mouse	6.75	-
	mouse (38.8 °C)[a]	6.6	-
	mouse (40.4 °C)[a]	5.17	-
	mouse (27.9 °C)[a]	10.21	-
Hydroxide	mouse	2.53	-

[a] Body temperature.

8.1.4.2. Central nervous system effects

Navazio et al. (1961) observed that characteristic toxic signs were not observed in rats until the ammonia concentration in the blood was double that of basal values (attained within 8 - 10 min of an ip injection of 601 mg ammonium acetate/kg body weight). No substantial increase in brain-ammonia was observed. However, when the blood-ammonia concentration reached more than 20 times the basal value, there was a sudden rise in the ammonia concentration in the brain, which reached a maximum of approximately 100 mg ammonia-nitrogen/kg (wet weight), between 10 and 26 min after injection. Muscular contractions with occasional tetanic spasms, and then coma occurred, when the concentration of ammonia in the brain reached approximately 50 mg/kg. Although the animals started to recover from the comatose state approximately 70 min after onset, blood- and brain-ammonia concentrations did not return to basal levels until 2 h after the injection of ammonium acetate.

However, other authors observed an immediate increase in brain-ammonia after ip injection of ammonium acetate (Torda, 1953; du Ruisseau et al., 1957; Salvatore et al., 1963). These authors found dramatic increases in the brain-ammonia content, 2 - 5 min after the injection of ammonium acetate. Salvatore et al. (1963) suggested that there was no critical blood-ammonia concentration for diffusion through the blood-brain barrier.

Ammonia has been shown to be more highly toxic at elevated body temperature, whereas hypothermia affords marked protection (Schenker & Warren, 1962). The LD_{50} values for ammonium chloride in the mouse, at various body temperatures, are shown in Table 19. The increased toxicity of ammonia at elevated body temperature was suggested to be due to a direct metabolic effect of hyperthermia on the brain, unrelated to dehydration or stress.

8.1.4.3. Effects on the heart

Intravenous LD_{50} values for ammonium carbamate, ammonium carbonate, and ammonium bicarbonate have been determined in mice by Wilson et al. (1968a,b) (Table 19). The physiological effects of the injected ammonium compounds in dogs and sheep were also investigated. Electrocardiograms recorded during the toxic syndrome indicated that the animals died from ventricular fibrillation due to a direct effect of ammonia on the heart. These findings were in agreement with the effects noted by Berl et al. (1962) during the iv infusion of ammonium chloride in cats when electrocardiograms were altered in a complex manner. However, Warren & Nathan (1958) were unable to demonstrate any cardiotoxic effects of the ammonium compounds in mice and concluded that the toxicity syndrome was due primarily to a cerebral effect and not a direct effect on cardiac or skeletal muscle.

8.2. Short-Term Exposures

8.2.1. Inhalation exposure

When 48 rats were continuously exposed to 127 mg ammonia/m^3 (181 ppm) for 90 days, no abnormalities were found in organs or tissues. Inhalation of 262 mg ammonia/m^3 (374 ppm) for 90 days induced mild nasal irritation in about 25% of 49 rats and a mild leukocytosis in 4 of the rats. Continuous exposure to 455 mg/m^3 (650 ppm) resulted in the death of 50 out of 51 rats by the 65th day of exposure. All animals exhibited mild nasal discharge and laboured breathing. In a second study, rats, guinea-pigs, rabbits, and dogs were continuously exposed to 470 mg/m^3 (671 ppm) for 90 days. Thirteen out of 15 rats and 4 out of 15 guinea-pigs died. Marked eye irritation was noted in rabbits and dogs, with corneal opacities in about one-third of the rabbits. At autopsy, all test animals examined had more extensive focal or diffuse interstitial inflammatory processes in the lungs than the controls (Coon et al., 1970).

White rats were exposed to ammonia, by inhalation, at concentrations of 100 and 30 mg/m^3 (143 and 43 ppm) for 25 or 60 min, every 48 h, for a period of 3 months. Rats exposed to a concentration of 100 mg/m^3 (143 ppm) showed only a mild leukocytosis, with no significant differences from the control group with regard to oxygen consumption, neuromuscular excitation threshold, heart rate, blood-sugar, blood-residual nitrogen, and total serum-protein (Prokop'eva & Yushkov, 1975). In another study by Alpatov & Mikhailov (1963), the threshold level for toxic effects in a 2-month exposure was 40 mg/m^3 (57 ppm). Histological changes were seen in the lungs of the animals exposed to a concentration of 100 mg/m^3 (143 ppm), including small areas of interstitial pneumonia with signs of peribronchitis and perivasculitis. No changes were seen in other organs compared with those in the control group.

Broderson et al. (1976) exposed Sherman and Fisher rats to ammonia from natural sources, at an average concentration of 105 mg/m^3 (150 ppm) for 75 days, and to purified ammonia at 175 mg/m^3 (250 ppm) for 35 days. 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. Lesions decreased posteriorly.

Exposure to ammonia at concentrations of 17 - 175 mg/m^3 (25 - 250 ppm) increased the infectious effects of *Pasteurella multocida* in mice and *Mycoplasma pulmonis* in rats; this effect increased with the concentration of ammonia (Broderson et al., 1976; Richard et al., 1978a).

Richard et al. (1978a) selected a concentration of 350 mg/m^3 (500 ppm) for a study of effects of continuous exposure to ammonia after noting that general toxic effects, particularly on growth rate, were not found at 175 - 210 mg/m^3 (250 - 300 ppm). Young male specific-pathogen-free rats were age- and weight-matched with controls (27 per group) and exposed for up to 8 weeks. Nasal irritation began on the fourth 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 in

the controls. After 8 weeks, none of these inflammatory lesions were present.

In a study by Coon et al. (1970), 15 rats, 15 guinea-pigs, 3 rabbits, 2 dogs, and 3 monkeys were exposed to an ammonia concentration of 155 mg/m^3 (221 ppm) for 8 h/day, 5 days a week, for 6 weeks. Pathological effects were not observed in any of the species except for evidence of focal pneumonitis in the lungs of one of the monkeys. Exposure to 770 mg/m^3 (1100 ppm) for the same duration induced mild to moderate eye irritation and laboured breathing in the rabbits and dogs at the beginning of exposure, but these signs disappeared by the second week and no other signs of irritation or toxicity were noted. Autopsy findings included non-specific inflammatory changes in the lungs of the rats and guinea-pigs.

Male Sprague Dawley rats were studied to determine whether ammonia was absorbed through the lungs into the blood, and the subsequent effects on the blood pH, blood gases, and hepatic drug-metabolizing enzymes (Schaerdel et al., 1983). Rats were exposed to ammonia at 7 - 840 mg/m^3 (10 - 1200 ppm) for 1, 3, or 7 days. No significant changes were found in blood pH, pCO_2, or in the histological appearance of the lungs or trachea. Liver microsomal enzymes (ethyl-morphine- *N*-demethylase and cytochrome P-450) showed only minor changes. Blood-ammonia levels increased in a linear fashion with increasing ammonia concentrations in air. A concentration of 70 mg/m^3 (100 ppm) or less produced only very small changes in blood-ammonia levels and had no measureable effects on any of the parameters studied.

8.2.2. Oral exposure

8.2.2.1. Histopathological effects

Rats given ammonium salts, for periods ranging up to 90 days, did not appear to sustain renal damage (Freedman & Beeson, 1961; Gupta et al., 1979). Twelve adult male Sprague Dawley rats were given ammonium chloride in the drinking-water at 16 g/litre, for up to 3 weeks. Urinalysis did not reveal any evidence of renal injury, and no gross or histological "abnormalities" of the kidney were seen at autopsy (Freedman & Beeson, 1961). Glutaminase activity per kg of kidney increased with duration of exposure; this is a physiological adaptation to acidosis. Another group of 10 rats, similarly treated and then given ammonium chloride in the drinking-water at 10 g/litre, for an additional 2.5 months also did not show any gross or microscopic renal abnormalities. Assuming the rats weighed 250 g and consumed 25 ml of water per day, they could have ingested as much as 1.6 g/kg body weight per day while drinking the ammonium chloride at 16 g/litre and 1.0 g/kg body weight per day while drinking a level of 10 g/litre. However, actual intake may have been lower, because ammonium chloride is known to affect the appetite and may render the water less palatable. When ammonium chloride was given at 20 g/litre, food and water consumption were drastically reduced.

In Table 20, selected studies are presented on ammonium salts other than the chloride. In general, at low doses, no detrimental effects were observed when rats and pigs were exposed to the NH_4 salts listed.

In a 90-day study by Gupta et al. (1979) on rats, there was not any evidence of renal damage. Ammonium sulfamate ($NH_4SO_3NH_2$) as a 100 g/litre solution was given orally at rates of 100, 250, or

5000 mg/kg body weight per day, 6 days a week for 30, 60, or 90 days to adult female rats (ITRC colony-bred albino) and to weanling male and female rats (20/sex per age per dose level). Under certain conditions, the sulfamate ion is hydrolysed to bisulfate ion and ammonia; however, it is unclear whether, and to what extent, hydrolysis occurs in the rat intestine. Equivalent doses, listed in Table 20, were calculated on the assumption that no hydrolysis occurs. The effects of the anion, sulfamate and/or sulfate, on the action of ammonium is a matter of conjecture. Ammonium sulfamate would be expected to produce metabolic acidosis on the basis of its structure, but this has not been verified experimentally. The general health of both treated and control rats was good, and there were no significant differences in mean body weights throughout the study, except for a slight depression in the highest-dose adult females at 60 and 90 days. Food and water consumption were unaffected, except in the highest dose weanlings of both sexes, which consumed less food and drank more water than control weanlings. Interim sacrifice of 6 animals/sex per dosage group was performed for haematological and histological examination. There were no significant differences in haematological values (packed cell volume, haemoglobin concentration, total red cell count, total and differential white cell count). Relative organ weights in all treated groups did not differ significantly from those in the controls. Histological examination of the kidney, liver, lung, stomach, heart, spleen, thyroid, adrenal, gonads, intestine, and lymph nodes did not reveal any abnormalities, except slight hepatic fatty degeneration in one adult female at 90 days.

Table 20. Dose-response data for short-term oral administration of ammonium com

Ammonium salt	Species, No/group, sex	Mode of administration	Duration	Daily Intake[a] (mg/kg body weight)	Daily Intake[a] (mg NH_3/kg body weight)[b]
Sulfamate	rat, albino, 20 adult female, 20 weanling male, 20 weanling female per dose	orally as 10% solution; not specified whether given by gavage	30, 60, 90 days	100; 250	15.0; 37.3
				500	74.8

Table 20. (contd.)

Ammonium salt	Species, No/group, sex	Mode of administration	Duration	Daily Intake[a] (mg/kg body weight)	Daily Intake[a] (mg NH_3/kg body weight)[b]
Diammonium citrate	pig, male 22.5 kg, 3-4/group	3.75% diammonium citrate in diet containing 6.4% crude protein	28 days	1820	274
Diammonium phosphate and diammonium citrate as equimolar mixture	pig, 27.3 kg 3 males and 3 females/ group	basal diet contained 16% protein; 0,5,10, or 20% of dietary N was replaced by N from equimolar mixture of designated NH_4+ salts	81 days	820	161 (10% replacement)
			81 days	1600	322 (20% replacement)

[a] Estimated for all studies except Gupta et al. (1979).

[b] Equivalent intake expressed as ammonia (NH_3).

Treatment of virgin female rabbits with ammonium carbonate, chloride, hydrophosphate, sulfate, or hydroxide at 0.1 - 0.2 g/kg body weight, orally, on alternate days, for periods of 3 weeks separated by 1-week intervals of no treatment, was associated with enlargement of the ovaries, follicle maturation, and formation of corpora lutea (Fazekas, 1949). There was also enlargement of the uterus, hypertrophy of the teats, and secretion of milk.

Rabbits (10 - 80 per group) were given 0.1 - 0.2 g/kg body weight of an ammonium salt in 100 - 150 ml of drinking-water, twice daily. One hundred and sixty rabbits (96 female, 64 male) received, twice daily, 50 - 80 ml of a 0.5% ammonium hydroxide solution (~135 - 210 mg/kg body weight), in gradually increasing doses. The chemicals were given for periods of 3 weeks separated by 1-week intervals of no treatment. In a related study, similar treatment of rabbits with ammonium chloride or ammonium sulfate resulted in fluctuations in serum-calcium and -phosphorus levels (Fazekas, 1954a).

8.2.2.2. Effects of ammonium as a dietary nitrogen supplement

Ammonia from ammonium salts can stimulate the growth of animals on diets deficient in non-essential amino acids or restricted in protein content. Weanling rats, given an artificial diet comprising essential amino acids, B vitamins, vitamin C, salts, and glucose for 21 days, grew poorly. Rats given a similar diet in which some of the glucose was replaced with 8.6% ammonium acetate (equivalent to 15.7 g nitrogen/kg diet) showed a dramatically improved weight gain (Birnbaum et al., 1957). The food consumption/weight gain ratio (feed/gain ratio) was also improved in animals receiving the ammonium salt.

Similar results were obtained with young male pigs given a more

natural basal diet restricted in non-essential amino acids and containing the minimum requirement of essential amino acids (Kagota et al., 1979). The crude protein level of this basal diet was 6.4%. Three pigs fed the basal diet supplemented with 3.75% diammonium citrate had a significantly greater weight gain and a slightly lower feed/gain ratio than 4 pigs fed the basal diet (the diets were made isocaloric by adjusting carbohydrate). No significant differences in the packed cell volume or ammonia-nitrogen level of the blood or in total protein concentration of plasma were found between the 2 groups. Plasma- and urine-urea-nitrogen were increased in the pigs fed the diet supplemented with diammonium citrate. Autopsy of the pigs after 28 days on the basal or ammonium citrate diets did not reveal any lesions or abnormalities or any differences in the percentage of carcass meat and fat. The average daily food intake for pigs receiving diammonium citrate was 1.44 kg, which corresponds to 54 g diammonium citrate per day. Using a body weight midway between the initial (22.5 kg) and final (36.7 kg) body weights, the mean intake of diammonium citrate can be estimated to be 1.82 g/kg body weight, per day, or 16.1 mEq NH_4+/kg body weight, per day.

Addition of ammonium salts to diets with a higher protein content (10% crude protein) did not produce significant changes in weight gain in rats or pigs (Clawson & Armstrong, 1981). Similarly, no significant changes in weight gain occurred in pigs, when up to 10% of the nitrogen from crude protein (diet = 16% crude protein) was replaced with nitrogen from ammonium salts (Wehrbein et al., 1970). Replacement of 20% of the dietary nitrogen with ammonium salts slightly decreased body weight gain in the pigs; food consumption and BUN also decreased. The 20% replacement diet contained 1.54% diammonium phosphate and 2.07% diammonium citrate for a total of 3.61% ammonium salts and was fed for 81 days. The estimated mean intake was 0.70 g diammonium phosphate and 0.94 g diammonium citrate/kg body weight per day, giving a total of 18.9 mEq NH_4+/kg body weight per day.

8.2.3. Dermal exposure

There is no information regarding systemic toxicity from short-term dermal exposure to ammonia or ammonium compounds.

8.3. Skin and Eye Irritation; Sensitization

Ammonia in the form of a gas, an anhydrous liquid boiling at low temperatures, or an aqueous solution is a recognized skin and eye irritant. Most of the information is human clinical data and is described in section 9.

Ammonia, partly because of its lipid solubility, penetrates the corneal membrane rapidly (NRC, 1979). In rabbits, conjunctival oedema with ischaemia and segmentation of limbal vessels were noted within 30 min. By 24 h, there was a reduction in the mucopolysaccharide contents of the corneal stroma, and extensive polymorphonuclear infiltration and anterior lens opacities were apparent. Aqueous levels of glucose and ascorbate and intraocular pressure were depressed (NRC, 1979). In rabbits with corneal burns, neovascularization occurred after 1 week, but it was delayed in animals with corneal and limbal burns. Complications of severe burns included symblepharon, pannus, pseudopterygia, progressive or recurrent corneal ulcerations leading to perforations, permanent corneal opacity, corneal staphyloma, persistent iritis, phthisis bulbi, secondary glaucoma, and dry eye (NRC, 1979).

Ammonium persulfate is a recognized skin sensitizer for human

beings. There are no data on its sensitization potential in animal models.

8.4. Long-Term Exposures

8.4.1. Inhalation exposure

Weatherby (1952) exposed 12 guinea-pigs to an ammonia concentration of about 119 mg/m^3 (170 ppm). Chamber concentrations ranged from 98 to 140 mg/m^3 (140 to 200 ppm) for 6 h/day, 5 days a week, for up to 18 weeks. There were no significant findings at autopsy in 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 suggested 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 casts. The cells of the adrenal glands were swollen and the cytoplasm in some areas had lost its normal granular structure.

Coon et al. (1970) conducted studies in which rats, guinea-pigs, rabbits, dogs, and monkeys were continuously exposed (24 h/day, 7 days per week) to ammonia. No signs of toxicity were seen in any species following continuous exposure to ammonia at a concentration of 40 mg/m^3 (57 ppm) for 114 days, and gross and microscopic examination did not reveal any lung abnormalities.

8.4.2. Oral exposure

The administration of ammonium carbonate, chloride, sulfate, hydrophosphate, acetate, lactate, or hydroxide to a total of 296 rabbits for 3 - 16 months resulted in enlargement of the parathyroids (Fazekas, 1954a). Administration of sodium dihydrophosphate, sodium ammonium phosphate, calcium chloride, hydrochloric acid, acetate acid, or lactic acid gave similar results (Fazekas, 1954a).

Rabbits given 0.1 g ammonium hydroxide/kg body weight (as a 0.5 - 1.0% solution) by gavage, initially, on alternate days and then daily for up to 17 months, had enlarged adrenal glands (Fazekas, 1939). An initial fall in blood pressure of 2.67 - 4.00 kPa (20 - 30 mmHg) was followed by a gradual rise to 1.33 - 4.00 kPa (10 - 30 mmHg) above the baseline, after several months of treatment.

The results of studies on rats, rabbits, and dogs indicate that long-term administration of ammonium chloride can induce osteoporosis (Seegal, 1927; Bodansky et al., 1932; Jaffe et al., 1932a,b; Barzel & Jowsey, 1969; Barzel, 1975). During prolonged metabolic acidosis, the release of bone mineral by resorption is thought to provide additional buffering capacity, sparing bicarbonate.

8.5. Reproduction, Embryotoxicity, and Teratogenicity

Charles & Payne (1966b) studied the effects of graded concentrations of atmospheric ammonia on the performance of laying hens. At 18 °C, ammonia at 73.5 mg/m^3 (105 ppm) significantly reduced egg production, after 10 weeks of exposure. No effects were observed on egg quality. Food intake was reduced and weight gain was lower. No recovery in egg production occurred when the treated groups were maintained for an additional 12 weeks in an

ammonia-free atmosphere. Similar results were observed at 28 °C, under the same conditions. Earlier work had indicated that egg quality could be affected by ammonia exposure (Cotterill & Nordskog, 1954). Freshly-laid eggs were exposed to various

concentrations of ammonia in a desiccator for 14 h at room temperature and then moved to normal atmosphere for another 32 h at 50 °C, before examination. There was evidence of absorption of ammonia into the eggs and significant impairment of interior egg quality, as measured by Haugh units, pH, and transmission of light.

The interaction of ammonium chloride with other teratogenic agents has been investigated in 2 studies. Goldman & Yakovac (1964) used ammonium chloride to investigate the role of metabolic acidosis in salicylate-induced teratogenesis in Sprague Dawley rats. Beginning on day 7 of gestation, rats received either 0.17 mol/litre (0.9%) ammonium chloride in the drinking-water, a single subcutaneous injection of salicylate, or both. Ammonium chloride alone inhibited fetal growth, but was not teratogenic. However, when administered with salicylate, ammonium chloride significantly increased maternal and fetal mortality and the fetal anomaly rate (dorsal midline, ventral midline, and eye defects) compared with that due to salicylate alone. These effects were attributed to acidosis and not to ammonia. However, it has been shown by Miller (1973) that the addition of 0.5% ammonium chloride to the 5%-glucose drinking-water of fasting, pregnant CFW mice significantly reduced the incidence of fast-induced cleft palate in the progeny.

8.6. Mutagenicity

Ammonium sulfate was reported to be non-mutagenic in the *Salmonella* and *Saccharomyces* systems (Litton Bionetics, Inc., 1975). Demerec et al. (1951) tested ammonia for its ability to induce back-mutations from streptomycin dependence to non-dependence in *Escherichia coli* and found it to be mutagenic, but only in treatments that left less than 2% survivors. Iwaoka et al. (1981a,b) showed that extraction of ingredients from fried hamburger and refrigerated biscuit products with ammonium hydroxide or ammonium sulfate increased the mutagenic activity in *S. typhimurium* TA98 and TA1538, compared with sodium sulfate extraction. This suggests that ammonium salts may, in some way, influence the mutagenic activity of some agents, or may themselves be responsible for false positive findings (Iwaoka et al., 1981a). However, it is also possible that ammonium salts extract mutagenic components from foods more efficiently.

Lobashov & Smirnov (1934) and Lobashov (1937) found ammonia to have a mutagenic effect on *Drosophila.* Ammonia showed slight mutagenic activity in studies in which survival of *Drosophila* was lower than 2% after treatment. In studies by Auerbach & Robson (1947), when *Drosophila* was exposed to ammonia vapour in small glass containers, 0.5% sex-linked lethals were observed.

It was reported by Rosenfeld (1932) that ammonia induced clumping of chromosomes, arrest spindle formation, and induce polyploidy in chick fibroblasts *in vitro.*

There are no data to show that ammonia is mutagenic in mammals.

8.7. Carcinogenicity

There is no evidence indicating that ammonia is carcinogenic.

Gibson et al. (1971) observed that animals treated with ammonia developed inflammatory lesions of the colon. It has been suggested that cell proliferation may increase errors in DNA replication (Zimber, 1970; Zimber & Visek, 1972), activate oncogenic agents present in sub-threshold doses (Anderson et al., 1964), and even unmask latent changes in the genetic material caused by mutagenic agents (Visek et al., 1978).

In a study on male Sprague Dawley rats (Topping & Visek, 1976), there was no evidence that ammonia increased the incidence of tumours with increased protein intake. Development of lung tumours was observed in CFLP mice treated intra-gastrically with diethyl pyrocarbonate and ammonia, neither of which induces cancer independently in animals. However, this may have resulted from a carcinogenic substance, possibly urethane, formed *in vivo* from diethyl pyrocarbonate in the presence of ammonia (Uzvolgyi & Bojan, 1980).

Life-long ingestion of ammonium hydroxide in the drinking-water by Swiss and C3H mice did not produce any carcinogenic effects, and had no effect on the spontaneous development of adenocarcinoma of the breast in C3H females, a characteristic of this strain (Toth, 1972).

Ammonium chloride and ammonium tungstoantimonate and related compounds have been shown to have an inhibitory effect on malignant cells (Phillips, 1970; Anghileri, 1975; Flaks & Clayson, 1975; Flaks et al., 1973; Sof'ina et al., 1978).

8.8. Factors Modifying Effects

8.8.1. Synergistic effects

Stevens et al. (1975) raised weanling rats for 3 - 6 weeks on a protein-deficient diet. Subsequent challenge by a large, intraperitoneally-injected dose of ammonium chloride indicated that severe protein deprivation increases vulnerability to ammonia poisoning in comparison with control groups not prefed protein-deficient diets.

Dalhamn (1963) employed a technique in rabbits in which an air pollutant mixture was drawn into the nostrils and out through a tracheal cannula. The effects of ammonia alone and ammonia mixed with (and presumably adsorbed on) carbon particles, on the beating rate of tracheal cilia was examined. Ammonia alone had to be administered at a high concentration (1400 mg/m^3 or 2000 ppm) to produce a tracheal concentration of 70 mg/m^3 (100 ppm). The reduction in beating rate was not substantially altered by the addition of carbon particles at 2 mg/m^3.

Simultaneous injection of ammonium salts and a fatty acid into rats or cats produced coma at lower plasma levels of ammonia and free fatty acids than a single injection of either compound (Zieve et al., 1974).

Impaired handling of an ammonium load has been observed in animals with hepatic dysfunction. Elevated levels of ammonia or urea have been reported in dogs with experimentally-bypassed livers (section 7.1.2). The importance of the kidney in detoxification of an ammonium load is apparent in the studies of Lotspeich (1965) (section 8.1.2.2), where an additive hypertrophic effect of ammonium chloride and unilateral nephrectomy was observed on the remaining kidney. Increased sensitivity to ammonia toxicity was found in young rats (Benyajati & Goldstein, 1975) and in rats that

had been castrated, adrenalectomized, or thymectomized (Paik et al., 1975).

8.8.2. Antagonistic effects

L-arginine and the related amino acids, ornithine and aspartic acid, substrates from urea synthesis, have been reported to exert a protective effect against acute ammonia poisoning in rats, dogs, and cats (Morris & Rogers, 1978; NRC, 1979).

High-protein diets exert an antagonistic effect on the toxicity of ammonium salts, unless liver dysfunction is present (section 7.1.2.4). In addition, an increase in toxic response has been observed with increase in age due to the adaptive response of glutaminase activity in rats to ammonia detoxication during acidosis.

8.9. Mechanisms of Toxicity

Hypotheses proposed for the mechanism of ammonia toxicity include impaired decarboxylation of pyruvic acid (McKhann & Tower, 1961), NADH depletion slowing down the generation of ATP (Worcel & Erecinska, 1962), depletion of alpha-ketoglutarate resulting in impairment of the Krebs cycle (Bessman & Bessman, 1955; Fazekas et al., 1956; Warren & Schenker, 1964); depletion of ATP due to glutamine formation by the glutamine synthetase (EC 6.3.1.2) system (Warren & Schenker, 1964; Nakazawa & Quastel, 1968), stimulation of membrane ATPase (EC 3.6.1.8) producing increased nerve cell excitability and activity (Hawkins et al., 1973), and depletion of ATP causing a decrease in cerebral acetylcholine (Braganca et al., 1953; Ulshafer, 1958).

9. EFFECTS ON MAN

9.1. Organoleptic Aspects

9.1.1. Taste

Campbell et al. (1958) determined the taste threshold concentration for ammonia in redistilled water with 21 - 22 subjects in "difference tests of the triangle type". At ammonia concentrations of 26, 52, and 105 mg/litre, the percentages of correct identifications were 61.9, 71.4, and 85.7, respectively.

Defining the threshold concentration as the level at which correct identification is 50% greater than that expected by chance, the taste threshold of ammonia was determined to be around 35 mg/litre. However, this definition of the threshold concentration seems to be somewhat arbitrary and McBride & Laing (1979) have reported significant positional bias in using the triangle test to determine taste thresholds. Furthermore, the triangle test is not intended to mimic environmental exposures in which the taste thresholds could be substantially higher. It seems reasonable to conclude only that the palatability of water is not likely to be significantly affected by total ammonia levels of $\leq$ 35 mg/litre (as NH_3), but will be affected at higher levels.

9.1.2. Odour

Odour thresholds reported in the literature may vary according to the definition of odour response, mode of presentation of the stimulus, chemical purity of the agent used, and the number of subjects and trials in the study. The detection threshold for ammonia, defined as the concentration that produces the first

detectable difference in odour over background, was reported to be 37 mg/m^3 (DallaValle & Dudley, 1939). This reference, though often cited, gives no information on the study design from which this number was derived.

The considerable variability in threshold data prompted work by Leonardos et al. (1969), who used a standardized procedure to determine recognition thresholds rather than detection thresholds for 53 chemicals. The odour threshold was defined as the first concentration at which all four panel members (trained odour analysts) were able to recognize the characteristic odour of the chemical. The panel tested only one chemical per day. Concentrations examined were multiples by 10 of 0.7, 1.47, and 3.3 mg/m^3 (1, 2.1, and 4.6 ppm). The recognition threshold for the odour of ammonia was 32.6 mg/m^3 (46.8 ppm).

The results of several other studies suggest that human beings can detect ammonia at much lower levels. Stephens (1971) reported 2.7 mg/m^3 (3.9 ppm) as the lowest concentration producing an odour response, when a contaminated air stream and a reference stream were compared by sniffing (number of subjects was not reported). A report by Saifutdinov (1966), of an olfactory threshold of 0.55 - 0.50 mg/m^3 for the most sensitive of 22 subjects, did not include sufficient detail to evaluate this excessively low estimate. Carpenter et al. (1948) stated that "a group of 8 persons found that the least odour they could detect on entering a room containing various concentrations" was 0.7 mg/m^3 (1 ppm) ammonia. Again, no other details were given.

The best estimates of the thresholds at which ammonia can be expected to be detected by taste and odour are 35 mg/litre (as NH_3) and 35 mg/m^3, respectively. Sensitive individuals may detect concentrations an order of magnitude lower.

9.2. Clinical and Controlled Human Studies

9.2.1. Inhalation exposure

The severe effects resulting from acute exposure to ammonia have been described in case reports of accidents involving groups of people or individuals. There are no data on actual levels of ammonia in air during such accidents, but estimates have been made (Yahagi et al., 1959; Takahashi et al., 1984; NRC, 1979).

Exposure to an ammonia concentration of 280 mg/m^3 (400 ppm) has been reported to produce immediate throat irritation; 1200 mg/m^3 (1700 ppm) to produce cough; 1700 mg/m^3 (2400 ppm) to be life-threatening, and 3500 - 7000 mg/m^3 (500 - 10 000 ppm) to cause a high mortality rate (Patty, 1963; Helmers et al., 1971).

A burning sensation in the eyes, nose, and throat, as well as respiratory distress accompanied by lachrymation, coughing, and an increase in respiratory rate are some of the irritant effects of ammonia (Caplin, 1941). Chest X-rays are generally normal in such mild cases (Watson, 1973; Close et al., 1980). More severe respiratory effects include laryngeal and pulmonary oedema and bronchopneumonia (Slot, 1938; Caplin, 1941; Levy et al., 1964; Taplin et al., 1976; Flury et al., 1983). The signs and symptoms are generally reversible, but chronic bronchitis and bronchiectasis have been reported (Slot, 1938; Sugiyama et al., 1968; Taplin et al., 1976; Close et al., 1980).

In cases with a lethal outcome, the cause of death has been severe lung damage and secondary cardiovascular effects (Slot, 1938; Mulder & van der Zalm, 1967).

There have also been some studies on volunteers exposed to ammonia under laboratory conditions. Some of these studies are summarized in Table 21.

Silverman et al. (1949) reported on 7 human volunteers exposed to a concentration of 300 mg/m^3 (500 ppm) ammonia for 30 min using an oral-nasal mask. All 7 experienced upper respiratory irritation, which lasted up to 24 h in 2 of the volunteers. Two subjects experienced marked lachrymation, in spite of the exposure being by oro-nasal mask. The average respiratory minute volume increased markedly compared with control values, and in the 5 subjects in which minute-by-minute expired volumes were measured, there was a marked cyclical variation in minute volume with a period of 4 - 7 min. No coughing was noted.

After exposure, respiratory minute volumes fell to levels below the pre-exposure rate, but returned to pre-exposure values within 5 min of exposure. Ammonia retention decreased progressively until an equilibrium of 24% retention (ranging from 4 to 30%) was reached at approximately 19 min (range 10 - 27 min). The indices of nitrogen metabolism (BUN, NPN, urine-urea, and urine-ammonia) remained normal. The carbon dioxide combining power did not change.

Verbeck (1977) assessed respiratory function in 16 volunteers following exposure to 35, 56, 77, and 98 mg ammonia/m^3 (50, 80, 110, and 140 ppm) for 0.5, 1, and 2 h. 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%. Subjective variables, including smell, taste, irritation of the eyes, nose, or throat, urge to cough, headache, and general discomfort were monitored every 15 min and ranked by 8 experts and 8 non-experts (students) on a scale from 0 to 5. Subjective responses were ranked higher by the non-experts. A concentration of 77 mg/m^3 (110 ppm) was tolerated for 2 h, but at 98 mg/m^3 (140 ppm), all the subjects left the chamber because the exposure was intolerable.

Respiratory responses to ammonia during exercise were examined by Cole et al. (1977). Eighteen males, aged 18 - 39 years, had 2 periods of exercise in 3 consecutive half-day sessions. Mean exposure levels were 71, 144, 106, and 235 mg/m^3 (101, 206, 151, and 336 ppm). Ventilation minute volume and total volume decreased, and mean respiratory frequency increased at exposure levels $\geq$ 106 mg/m^3 (151 ppm).

In a study by MacEwen et al. (1970), 6 volunteers were exposed to ammonia concentrations of 21 and 35 mg/m^3 (30 and 50 ppm) for 10 min. The irritation was rated subjectively on a scale of 0 - 4. At 35 mg/m^3 (50 ppm), irritation was rated as "moderate" by 4 of the volunteers, while 1 individual reported no detectable irritation. None of the volunteers found the irritation at 35 mg/m^3 (50 ppm) to be "discomforting" or "painful".

The irritation threshold for ammonia was examined in 10 human volunteers by Industrial Bio-Test Laboratories, Inc. (1973). The subjects were exposed to 4 different concentrations of 22, 35, 50, and 94 mg/m^3 (32, 50, 72, and 134 ppm) for 5 min. The frequency of

positive findings for the 10 subjects was as follows: at 22 mg/m^3, 1 subject complained of dryness of the nose; at 35 mg/m^3, 2 subjects experienced dryness of the nose; at 50 mg/m^3, 3 subjects had eye irritation, 2 had nasal irritation, and 3 had throat irritation; at 94 mg/m^3, 5 subjects demonstrated signs of lachrymation, 5 had eye irritation, 7 had nasal irritation, 8 had throat irritation, and 1 complained of chest irritation.

Table 21. Effects of inhaled ammonia in human volunteers

Number	Concentration (mg/m^3)	Duration (min)	Effects and response	Reference
7	300	30	irritation of upper respiratory tract (7/7); increase in minute volume; no change in blood chemistry	Silverman et
16	35 - 98	30 - 120	10% increase in VC, FEV, and FIV; 98 mg/m^3 was not tolerated; 77 mg/m^3 was tolerated for 2 h	Verbeck (197
18	71 - 235	8 - 11	no effects noted during exercise at 71 mg/m^3 except slight irritation; decrease in ventilation minute volume and tidal volume and increase in respiratory frequency $\geq$ 106 mg/m^3	Cole et al.
6	35	10	"moderate irritation" (4/5)	MacEwen et a
10	22	5	dryness of the nose (1/10)	Industrial B
10	35		dryness of the nose (2/10)	Laboratories
10	50		eye irritation (3/10)	
10	94		throat irritation (8/10) eye irritation (5/10)	
6	17.5, 35, or 70+	2 - 6 h, 5 days/ week, for 6 weeks	increase in FEV_1, with increasing NH_3; adaptation to irritating effects	Ferguson et a

A controlled study on human volunteers was conducted by Ferguson et al. (1977) to evaluate the responses to inhaled ammonia at concentrations of 17.5, 35, and 70 mg/m^3 (25, 50, and 100 ppm). Six adults, not acclimatized to ammonia exposure, were divided into 3 groups, which were exposed for 2 - 6 h each day, 5 days per week, for 6 weeks. One pair of subjects was exposed only to 35 mg ammonia/m^3 for 6-h periods throughout the test. Subjects were examined daily for irritation of the eyes, nose, or throat, and periodically for pulse rate, respiration rate, pulmonary function (forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1)), blood pressure, neurological responses, and interference in task-performance ability. A statistical analysis of the results demonstrated that the only significant change among the vital functions measured was an increase in forced expiratory volume in 1 second (FEV_1) with increasing ammonia concentration. In addition, the rate of mild eye, nose, or throat irritations over the last 3 weeks of the test was significantly less than during the first 2 weeks. This indicated that an acclimatization process had occurred, with increased tolerance to the irritant effects of

ammonia developing with increasing time of exposure. Overall, the ammonia exposure produced 2 incidents of mild irritation (78 observations made) at 17.5 mg/m^3 (25 ppm), 22 incidents (198 observations made) at 35 mg/m^3 (50 ppm), and 11 incidents (84 observations made) at 70 mg/m^3 (100 ppm). Among control subjects, 4 irritation incidents were recorded during 45 observations. When ammonia concentrations exceeded 105 mg/m^3 (150 ppm), all subjects experienced lachrymation accompanied by dryness of the nose and throat.

In an inhalation study, the threshold for effects on respiration, skin electric potential, and the electroencephalogram was found to be 22 mg/m^3 (31 ppm) (Alpatov & Mikhailov, 1963; Alpatov, 1964).

Reports from industries with ammonia exposure indicate that irritative effects have appeared over a wide range of ammonia concentrations (Elkins, 1950; Vigliani & Zurlo, 1955; Mangold, 1971). A concentration of 88 mg/m^3 was called "definitely irritating" (Elkins, 1950), and "barely noticeable" eye irritation was reported at 3 mg/m^3 (Mangold, 1971).

Giguz (1968), in a study involving 140 subjects exposed to ammonia and nitrogen oxides, at concentrations not exceeding the "maximum permissible concentration" (20 mg/m^3), 3 h per day during 2 - 3 years of vocational training, demonstrated increased incidences of upper respiratory tract disease, compared with those in a control group of unexposed subjects.

9.2.2. Oral exposure

9.2.2.1. Effects of acute oral exposure

Cases of the ingestion of large doses of ammonia have been reported. When solutions of ammonia were ingested orally, a tissue-destructive caustic effect was noted for concentrated solutions, owing to their high pH. A solution of ammonia at a concentration of 100 g/litre, for example, has a pH of 12.5.

Oesophageal burns were reported in 25 cases of accidental ammonia ingestion (Hawkins et al., 1980). One child suffered a mild burn. Four adults had mild burns limited to the mucosa and nine adults had oesophageal burns that were moderate or more severe in nature. One adult female suffered from a complication of airway obstruction from supraglottic oedema. Oesophageal stricture from the ingestion of ammonia (100 g/litre) was reported by Vancura et al. (1980) (Table 19).

A fatal outcome after ingestion of a solution containing 24 g ammonia/litre was reported by Klendshoj & Rejent (1966). Autopsy showed haemorrhagic inflammatory changes in the oesophagus, stomach, and small intestine.

There are many reports on the effects of ammonium chloride, but since acidosis is caused by the chloride, such studies have little relevance for evaluating ammonia toxicity.

There are no data on acute effects of ingestion of ammonium compounds, other than the chloride.

9.2.3. Endogenous hyperammonaemia

9.2.3.1. Inborn errors of metabolism

These affect the uptake of ammonia rather than its rate of production.

Congenital deficiency of carbamyl phosphate synthetase I (EC 6.3.4.1.6), and, to a lesser extent, of other enzymes of the ornithine cycle, and several other metabolic disorders may lead to hyperammonaemia and various abnormal urinary constitutents. Hyperammonaemia may be lethal in new-born infants, may cause severe symptoms in infancy, or may cause chronic remittent symptoms in older children or adults (Hsia, 1974).

Clinical features in neonates may resemble those of hepatic coma and may be precipitated by protein-rich milk feeds. In older children, episodic vomiting, neurological disorders (including seizures) or coma are precipitated by high-protein foods.

9.2.3.2. Hepatic features

Varied and complex functions of the liver may fail progressively in chronic liver disease or rapidly in acute disorders. The syndromes differ in the extent to which portal blood from the intestine is shunted into the systemic venous circulation either by cirrhotic changes in hepatic vascular resistance or by surgical procedures to correct portal hypertension resulting from it.

In either case, a syndrome is recognised that comprises a spectrum of neurological features from irritability via inappropriate behaviour, tremors, hyperreflexia and generalised muscular rigidity, to delirium, stupor, convulsions and coma.

There is disagreement regarding the extent to which this syndrome is an expression of ammonia toxicity. On the one hand, over 90% of persons with the disorder have elevated levels of ammonia in the blood or cerebrospinal fluid; the condition may be induced in those with marginal liver function by the administration of ammonium salts or an intestinal haemorrhage (which leads to intestinal ammonia production greater than that from an equivalent amount of meat) and the condition may be treated by reducing intestinal ammonia production, by the administration of antibiotics to eliminate ammonia-producing flora. On the other hand, there is an imperfect correlation between the clinical state and the blood-ammonia level and the condition may occur in the presence of normal blood-ammonia levels.

It is unlikely that the syndrome resulting from the failure of an organ as complicated as the liver should be explicable in terms of a single metabolic component, but the similarity between hepatic failure and certain expressions of congenital hyperammonaemias suggests an important role of ammonia toxicity in the pathogenesis of the syndrome.

10. EVALUATION OF HUMAN HEALTH RISKS AND EFFECTS ON THE ENVIRONMENT

10.1. Atmospheric Exposure and Effects

10.1.1. General population exposure

Background levels of ammonia in community air are low in comparison with levels that have been established as safe in occupational settings, but there is considerable variabilty

according to the type of land use. In general, levels in areas with intensive livestock husbandry or high rates of manure application are in the range of 100 - 200 µg/m^3, levels in urban areas range from approximately 5 to 40 µg/m^3, while those in rural areas, without intensive manure production or use, range up to 10 µg/m^3. This is in contrast to odour thresholds of the order 10 000 µg/m^3, thresholds for irritation of the order 20 000 - 50 000 µg/m^3, and the estimated LC for man of 5000 - 10 000 mg/m^3. The LC_{50} estimation for the rat was 7 600 000 µg/m^3, for a 2-h exposure. In an occupational setting, workers did not voluntarily use respiratory protective devices at concentrations below about 500 000 µg/m^3. General ambient atmospheric levels are therefore of no concern in respect of discomfort or acute toxicity.

Ammonia is not mutagenic and long-term studies on both laboratory and farm animals have not shown any pathological effects at levels below 35 000 µg/m^3. Long-term toxic effects are unlikely, even at levels much higher than ambient levels, both generally and in the neighbourhood of ammonia-emitting systems. There is a lack of evidence regarding recent trends in global atmospheric ammonia concentrations.

10.1.2. Occupational exposure

Occupational problems are predominantly those of accidental exposure.

Though for certain groups in, for example, agriculture, the chemical industry, waste disposal and transport, occupational exposure levels may be very much higher than general, there is historical evidence that, even at levels significantly in excess of current occupational exposure limits, there was a low prevalence of adverse health effects.

The distribution kinetics of absorbed atmospheric ammonia suggest that the rise in blood-ammonia at a typical occupational exposure limit will be within the normal range of variation.

Thus, there is both historical and theoretical evidence that most recommended exposure limits are acceptable.

10.2. Exposure Through Water and Food

Ammonia will have a toxic effect, only if intake exceeds the capacity of mammals to detoxify ammonia. Unfortunately, there are no data permitting the evaluation of this capacity in healthy human beings or other terrestrial mammals. In addition, there is some evidence that the mode of intake may be a factor in the capacity of individuals to detoxify ammonia. Parenteral administration of ammonia results in patterns of metabolism and elimination that are markedly different from those seen in oral administration. Thus, the considerable body of data on the parenteral toxicity of ammonia is not of direct relevance to criteria for oral exposure. Similarly, because of the different kinetic patterns between oral and inhalation exposure to ammonia, as well as the highly irritant effects of ammonia on the lung, the available inhalation data on ammonia are not applicable to the estimation of an acceptable daily intake (ADI) for ingestion. However, it would be possible to define a clearly undesirable level of oral exposure to total ammonia (NH_3 and NH_4+) as well as a level that is clearly tolerable. The range between these 2 levels would exceed the range within which an ADI could be established.

The amount of excess ammonia (i.e., over and above the amount normally produced in the body) that can be safely ingested and assimilated is difficult to define. In short-term (28 - 90 days) studies carried out on rats and pigs, no adverse effects were reported at higher levels of ammonia intake (75 - 545 mg NH_3/kg body weight per day) in the form of sulfamate, phosphate, citrate, or chloride.

The effects attributed directly to elevated ammonium ion levels are acute pulmonary oedema and central nervous system (CNS) toxicity, depression of appetite due to a direct effect of the ammonium ion on the brain, and promotion of growth via the use of ammonium salts as a source of non-essential nitrogen under certain circumstances.

Some effects (such as renal growth and demineralization of bone) arising from the administration of ammonium chloride seem to be secondary effects of acidosis.

Surveys of total ammonia (NH_3 + NH_4+) concentrations in surface waters indicate an average of < 0.18 mg/litre in most areas, and 0.5 mg/litre in waters near large metropolitan areas (Wolaver, 1972; US EPA, 1979a). Levels in ground water are usually low, since ammonia is generally immobile in soil. Ammonia is practically absent when drinking-water is chlorinated.

Ammonia is a negligible natural constituent of food, but ammonium compounds are added in small amounts (< 0.001 - 3.2%) to various foods as stabilizers, leavening agents, flavourings, or for other purposes. The daily human intake from these sources is estimated to be 18 mg as NH_3.

10.3. Ocular and Dermal Exposure

Ammonia in aqueous solution or in contact with body fluids is alkaline and causes burns or inflammation of eyes or skin. The ocular irritation commonly experienced at atmospheric ammonia concentrations of > 20 mg/m^3 (MacEwen et al., 1970; Keplinger et al., 1973; Verberk, 1977) is readily reversible when exposure ceases, and may also be reduced by acclimatization (Ferguson et al., 1977).

Serious ocular damage normally occurs, only with a direct blast or splash contact with anhydrous or aqueous ammonia (Grant, 1974). Skin damage is reported to occur at concentrations of ~7000 mg/m^3 (NRC, 1979).

10.4. Accidental Exposure

High gaseous ammonia concentrations may be encountered locally, both in domestic and work-place environments, as a result of gaseous emissions and/or spillages of concentrated solutions, and respiratory (and skin and eye) injury may result. On a larger scale, spillage from stock or transport tanks or refrigeration plant of concentrated ammonia liquor or anhydrous ammonia would constitute a severe environmental insult and would cause serious injury to the people, animals, and plants in the vicinity. Because of its low density and short biopersistence, major spillages would be expected to disperse rapidly and not to persist in the environment.

10.5. Evaluation of Risks for the Environment

Environments that receive more ammonia than can be used may be

acidified and nitrogen-enriched. As a consequence of these physical-chemical changes, the structure and functioning of the ecosystem will be disturbed.

Ammonia plays an important role in the metabolism of all organisms as a nutrient at low concentrations, but becomes toxic at higher concentrations. For example, microorganisms both assimilate and generate ammonia as a part of natural nutrient cycling processes. High levels of ammonia may inhibit growth or survival of microbial organisms, including, at higher levels, nitrifying organisms.

10.5.1. The aquatic environment

Ammonia concentrations in the aquatic environment are variable, reflecting the proximity and nature of sources (section 4).

Where there are large numbers of people and animals in relation to the volume of surface waters and drainage flow, the load of nitrogen added to surface waters from sewage and industrial effluent is the predominant source and may lead to ammonia concentrations that constitute a significant local and/or regional environmental problem.

Otherwise, ammonia deposition contributes a major input. In surface waters that are poorly buffered, poor in nutrients, and hydrologically dependent on rainfall and/or snow melt, this may result in acidification and nitrogen enrichment resulting in marked changes in plant community structure with concommitant changes in the animal population structure.

The toxic effects on aquatic organisms are attributed to non-ionized ammonia (NH_3) rather than to ammonium ion levels. This is because non-ionized ammonia easily penetrates biological membranes, whereas ammonium ions require specialized transport processes.

There are similarities between the modes of the acute toxic action of ammonia in mammals and in fish: in the latter, however, environmental conditions (such as pH, ionic composition and concentration, temperature, and oxygen availability), which are more variable in water than in air, have a marked modulating effect.

Aquatic animals have a limited ability to detoxify ammonia and, therefore, the body load is dependent on ammonia concentrations in the water. Except in open oceans, exposure to environmental levels produces many chronic effects (including reduced growth, decreased survival, impaired reproduction) and may increase susceptibility to disease and also cause histopathological changes.

High levels of ammonia in aquatic systems are also toxic for plants. The detoxification of excessive ammonia places a severe strain on the carbohydrate metabolism of the plant which subsequently results in foliar injury, and growth effects, and thus may modify plant community composition.

Where the dominant species, be it fish or plant, is also sensitive to ammonia the effects on the whole ecosystem will be marked.

10.5.2. The terrestrial environment

The most common effect of exposure of plants to atmospheric ammonia is foliar injury. Prolonged exposure to high ambient

concentrations of about 75 µg/mg, such as occur in the vicinity of intensive livestock farms, adversely affects more susceptible species such as pine trees. The observed damage is the result of both direct and indirect effects due to, changes in soil, and secondarily, to increased susceptibility to disease and meteorological stress.

The data on ammonia toxicity for wildlife are very limited. There is no evidence that wildlife populations, in general, are at risk from ammonia, but, there may be secondary effects associated with changes in plant communities. Certain species of bats are able to withstand the very high ammonia levels found in caves where they live.

10.6. Conclusions

The major groups of organisms at risk from elevated ammonia levels are aquatic animals and terrestrial plants. There appears to be little danger for terrestrial animals, including man, except from acute accidental exposure.

10.6.1. General population

There are no data suggesting that present environmental levels of ammonia are hazardous for the general population. Only high-level accidental exposures from domestic sources and transportation and storage accidents pose an occasional acute health hazard.

10.6.2. Sub-populations at special risk

Groups likely to exhibit ammonia toxicity include those with hepatic or renal impairment, though, even in these cases, levels of exogenous ammonia are insignificant in comparison with endogeneous levels, so that, in the absence of any environmental exposure, such persons would still be affected. The mechanism is different in the two cases. Hepatic impairment limits the conversion of ammonia to urea, and renal failure, by increasing urea concentrations and its intestinal secretion, leads to increased endogeneous intestinal ammonia production.

There have been few studies on the chronic effects of ammonia inhalation. It can be speculated that subpopulations that have been found to be hyperreactive to other respiratory irritants (e.g., sulfur dioxide, particulates, ozone) may also be hyperreactive to ammonia. These subpopulations may include children, elderly persons with pre-existing cardiorespiratory symptoms, individuals with asthma or bronchitis, and those engaged in vigorous physical exercise (Calabrese, 1978). However, there is also some indication that previous exposure to low levels of ammonia may cause inurement to its effects (Ferguson et al., 1977).

10.6.3. Occupational exposure

Accidental exposures are the predominant problem (section 10.1.1.3). Otherwise, occupational exposure can be controlled by the application of most current occupational exposure limits and proper industrial hygiene.

10.6.4. Farm animals

Farm animals may be at risk, because of continous exposure under confined housing conditions resulting in high atmospheric levels of ammonia within the confinement areas; this applies particularly to cattle, swine, and poultry. Available reported

data provide a range of measured exposure levels of from 2 to 1400 mg/m^3. In winter months in colder climates, most of the measured concentrations exceeded the admissible upper limit of 35 mg/m^3 (50 ppm).

10.6.5. Environment

Environments with a low buffer capacity and poor in nutrients are susceptible to acidification and nitrogen enrichment by elevated ammonia loading; prolonged high ammonia-loading results in changes in both the structure and function of plant and animal communities. Levels of atmospheric ammonia necessary for the onset of these changes have not been established; however, changes in the structure of these communities have been observed where ammonia levels in the atmosphere were possibly up to 100 µg/m^3.

Plants use ammonia as a nutrient, but high levels can be toxic. Terrestrial plants show a susceptibility to reduced growth and reduced vitality, when exposed to levels as low as 75 µg NH_3/m^3 in the atmosphere.

Aquatic animals have a low capacity to detoxify ammonia. Acute effects on some fish have been demonstrated in laboratories at concentrations as low as 0.1 mg NH_3/litre and chronic effects at concentrations as low as 0.02 mg NH_3/litre. Thus, as ammonia levels in some waters are often similar to those shown to cause chronic effects in some fish, it would appear that these animals are at risk. Aquatic invertebrates are, in general, less sensitive to elevated ammonia levels in water.

Aquatic animals are at risk because of increases in ammonia concentrations in water systems, whereas some plant communities appear to be at risk from elevations in atmospheric ammonia loading.

11. RECOMMENDATIONS

11.1. Research Needs

1. Long-term monitoring of ammonia and other pollutants in water systems with different aquatic ecosystems.

2. Studies of the long-term effects of ammonia on terrestrial vegetation.

3. Studies of the global nitrogen balance to identify long-term trends.

4. Ecotoxicological studies to elucidate environmental effects.

5. Epidemiological studies in relation to ammonia exposure in order to make better hygiene recommendations.

6. Long-term experimental animal studies to establish a no-observed-adverse-effect level of exposure.

7. Studies on the role of ammonia in modifying physical and chemical conditions of soil and water systems.

8. More data are needed to assess accurately the relative contributions of various point and non-point sources of ammonia for surface waters.

9. Research into methods and their application directed towards reducing emissions from point sources.

10. Additional acute toxicity tests with salt-water fish and invertebrate species.

11. Life-cycle and early-life-stage tests with representative fresh-water and salt-water organisms from different families, with investigation of pH effects on chronic toxicity.

12. Fluctuating or intermittent exposure tests under a variety of exposure patterns on additional species.

13. Both acute and long-term tests at cold-water temperatures.

14. Studies on the effects of dissolved and suspended solids on acute and chronic toxicity.

15. More histopathological and histochemical research with fish, which would provide a rapid means of identifying and quantifying sublethal ammonia effects.

16. In fish, the relative concentration limits for both acclimatization and subsequent acute response need better definition and a more complete explanation.

REFERENCES

ABBAS, R. & TANNER, R.L. (1981) Continuous determination of gaseous ammonia in the ambient atmosphere using fluorescence derivatization. *Atmos. Environ.,* **15**: 277-281.

ABELOVICH, A. & AZOV, Y. (1976) Toxicity of ammonia to algae in sewage oxidation ponds. *Appl. environ. Microbiol.,* **31**(6): 801-806.

ADMIRAAL, W. (1977) Tolerance of estuarine benthic diatoms to high concentrations of ammonia, nitrite ion, nitrate ion and orthophosphate. *Mar. Biol.,* **43**(4): 307-315.

AGAMI, M., LITAV, M., & WAISEL, Y. (1976) The effects of various components of water pollution on the behaviour of some aquatic macrophytes of the coastal rivers of Israel. *Aquat. Bot.,* **2**(3): 203-213.

ALABASTER, J.S. & HERBERT, D.W.M. (1954) Influence of carbon dioxide on the toxicity of ammonia. *Nature (Lond.),* **174**(4426): 404.

ALABASTER, J.S., SHURBEN, D.G., & KNOWLES, G. (1979) The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon, *Salmo salar* L. *J. Fish Biol.,* **15**(6): 705-712.

ALLAN, I.R.H. (1955) Effects of pollution on fisheries. *Verh. Int. Ver. Theor. Agnew Limnol.,* **12**: 804-810.

ALLAN, I.R.H., HERBERT, D.W.M., & ALABASTER, J.S. (1958) A field and laboratory investigation of fish in a sewage effluent. *Fish. Invest.,* **6**(2): 1-76.

ALPATOV, I.M. (1964) [A study of gazeous ammonia toxicity.] *Gig. Tr. prof. Zabol.,* **2**: 14-18 (in Russian).

ALPATOV, I.M. & MIKHAILOV, V.I. (1963) [Inquires into the gaseous ammonia toxicity. Communication 1.] *Gig. Tr. prof. Zabol.,* **12**: 51-53 (in Russian)

ANALYTICAL QUALITY CONTROL (HARMONISED MONITORING) COMMITTTEE (1982) Accuracy of determination of ammoniacal nitrogen in river waters: analytical quality control in the harmonised monitoring scheme. *Analyst,* **107**(June): 680-688.

ANDERSON, B.G. (1948) The apparent thresholds of toxicity to *Daphnia magna* for chlorides of various metals when added to Lake Erie water. *Trans. Am. Fish Soc.,* **78**: 96-113.

ANDERSON, D.P., BEARD, C.W., & HANSON, R.P. (1964a) The adverse effects of ammonia on chickens including resistance to infections with Newcastle disease virus. *Avian Dis.,* **8**: 369-379.

ANDERSON, D.P., CHERMS, F.L., & HANSON, R.P. (1964b) Studies on measuring the environment of turkeys raised in confinement. *Poult. Sci.,* **43**: 305-318.

ANDERSON, K.B., SPARKS, R.E., & PAPARO, A.A. (1978) *Rapid assessment of water quality, using the fingernail clam,* Musculium transversum, Urbana, Illinois, Water Resources Center, University of Illinois, 115 pp (WRC Research Report No. 133).

ANGHILERI, L.G. (1975) Tumour growth inhibition by ammonium chloride-induced acidosis. *Int. J. clin. Pharmacol.,* **12**: 320.

ANTHONISEN, A.C., LOEHR, R.C., PRAKASAM, T.B.S., & SRINATH, E.G. (1976) Inhibition of nitrification by ammonia and nitrous acid. *J. Water Poll. Control Fed.,* **48**: 835-852.

APHA (1976) *Standard methods for the examination of water and wastewater,* 14th ed., Washington DC, American Public Health Association, American Water Works Association, Water Pollution Control Federation, 1193 pp.

API (1981) *The sources, chemistry, fate, and effects of ammonia in aquatic environments,* Washington DC, American Petroleum Institute, 145 pp.

APPELMAN, L.M., TEN BERGE, W.F., & REUZEL, P.G.J. (1982) Acute inhalation toxicity study of ammonia in rats with variable exposure periods. *Am. Ind. Hyg. Assoc. J.,* **43**(9): 662-665.

ARMSTRONG, D.A., CHIPPENDALE, D., KNIGHT, A.W., & COLT, J.E. (1978) Interaction of ionized and un-ionized ammonia on short-term survival and growth of prawn larvae, *Macrobrachium rosenbergii. Biol. Bull.,* **154**(1): 15-31.

ARNDT, F.L., DAY, D.L., & HATFIELD, E. (1979) Processing and Handling of animal excreta for refeeding. *J. anim. Sci.,* **48**: 157.

ASTM (1980) *Annual book of ASTM standards. Part 31. Water,* Philadelphia, Pennsylvania, American Society for Testing and Materials.

AUERBACH, C. & ROBSON, J.M. (1947) Tests of chemical

substances for mutagenic action. *Proc. R. Soc. Edinburgh Sec. Biol.,* **62B**: 284-291.

AVOT, YU.K., KANEP, V.V., SLUTSKER, D.S., & SHAFRAN, L.M. (1977) *[Medical and sanitary aspects of sailors' work,]* Riga, Zvaigzne, 132 pp (in Russian).

AXELROD, H.D. & GREENBERG, J.P. (1976) Determination of trace concentration of ammonia in the atmosphere using pyridine-pyrazolone reagent. *Atmos. Environ.,* **10**: 495-496.

BAIRD, R., BOTTOMLEY, J., & TAITZ, H. (1979) Ammonia toxicity and pH control in fish toxicity bioassays of treated wastewaters. *Water Res.,* **13**(2): 181-184.

BALL, I.R. (1967) The relative susceptibilities of some species of fresh-water fish to poisons. I. Ammonia. *Water Res.,* **1**(11/12): 767-775.

BARROW, C.S. & STEINHAGEN, W.H. (1980) NH_3 concentrations in the expired air of the rat: Importance to inhalation toxicology. *Toxicol. appl. Pharmacol.,* **53**: 116-121.

BARROW, C.S., ALARIE, Y., & STOCK, M.F. (1978) Sensory irritation and incapacitation evoked by thermal decomposition products of polymers and comparisons with known sensory irritants. *Arch. environ. Health,* **33**(2): 79-88.

BARTON, L.V. (1940) Toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide and sulphur dioxide gases. IV. Seeds. *Contrib. Boyce Thompson Inst.,* **11**: 357-363.

BARZEL, U.S. (1975) Effect of chronic ammonium chloride ingestion on parathyroid-hormone function. *Nephron,* **14**(15): 339-346.

BARZEL, U.S. & JOWSEY, J. (1969) Effects of chronic acid and alkali administration on bone turnover in adult rats. *Clin. Sci.,* **36**(3): 517-524.

BENEDICT, H.M. & BREEN, W.H. (1955) The use of weeds as a means of evaluating vegetation damage caused by air pollution. In: *Proceedings of the Third National Air Pollution Symposium, Pasadena, California, April 1955,* pp. 177-190.

BENNETT, W.F., PESEK, J., & HANWAY, J.J. (1964) Effects of nitrate and ammonium on growth of corn in nutrient solution sand culture. *Agric. J.,* **56**: 342-345.

BENYAJATI, S. & GOLDSTEIN, L. (1975) Renal glutaminase adaptation and ammonia excretion in infant rats. *Am. J. Physiol.,* **228**(3): 693-698.

BERL, S., TAKAGAKI, G., SCHWERIN, P., & WAELSCH, H. (1962) Metabolic compartments *in vivo:* Ammonia and glutamic acid metabolism in brain and liver. *J. biol. Chem.,* **237**: 2562-2569.

BESSMAN, S.P. & BESSMAN, A.N. (1955) The cerebral and peripheral uptake of ammonia in liver disease with an hypothesis for the mechanism of hepatic coma. *J. clin. Invest.,* **34**: 622-628.

BHATTACHARYA, A.N. & TAYLOR, J.C. (1975) Recycling animal waste as a feedstuff: A review. *J. anim. Sci.,* **44**: 1438.

BIDWELL, R.G.B. (1974) *Plant physiology,* New York, MacMillan, 197 pp.

BINSTOCK, L. & LECAR, H. (1969) Ammonium ion currents in the squid giant axon. *J. gen. Physiol.,* **53**: 342-361.

BIRNBAUM, S.M., WINTZ, M., & GREENSTEIN, J.P. (1957) Quantitative nutritional studies with water-soluble, chemically defined diets. III. Individual amino acids as sources of "non-essential" nitrogen. *Arch. Biochem. Biophys.,* **72**(2): 428-436.

BISWAS, S.D. & KLEINBERG, I. (1971) Effect of urea concentration on its utilisation, on the pH and the formation of ammonia and carbon dioxide in a human salivary sediment system. *Arch. oral Biol.,* **16**: 759-780.

BLOOMFIELD, R.A., GARNER, G.B., & MUHRER, M.E. (1960) Kinetics of urea metabolism in sheep. *J. anim. Sci.*, **19**: 1248.

BLOOMFIELD, R.A., KEARLEY, E.O., CREACH, D.O., & MUHRER, M.E. (1962) Ruminal pH and absorption of ammonia and VFA. *J. anim. Sci.,* **22**: 833 (Abstract).

BOCK, F., MICHELSON, I., BROSS, I.D., & PRIORE, R.L. (1974) Carcinogenic activity of smoke condensate from cigarettes with ammonium sulfamate-treated paper. *Cancer,* **33**: 1010.

BODANSKY, A., JAFFE, M.L., & CHANDLER, J.P. (1932) Serum phosphatase changes in ammonium chloride osteoporosis. *Proc. Soc. Exp. Biol. Med.,* **29**: 871-875.

BOER, W.M.J., DEN & BASTIAENS H. (1984) *[Effects of acidification on vegetation,]* Ministry of Agriculture and Fisheries, Ministry of Housing, Physical Planning and Environment, 82 pp (Report No. 84(2)) (in Dutch).

BOTHAST, R.J., LANCASTER, E.B., & HESSELTINE, C.W. (1973) Ammonia kills spoilage moulds in corn. *J. Dairy Sci.,* **56**: 241.

BOTHAST, R.J., LANCASTER, E.B., & HESSELTINE, C.W. (1975a) Effect of pH and substrate on growth. *Eur. J. appl. Microbiol,* **1**: 55-56.

BOTHAST, R.J., ADAMS, G.H., HATFIELD, E.E., & LANCASTER, E.B. (1975b) Preservation of high-moisture corn: a microbiological evaluation *J. Dairy Sci.,* **58**: 386-391.

BOYD, E.M. & SEYMOUR, K.G.W. (1946) Ethylenediamine dihydrochloride or "chlorethamine". II. Untoward and toxic reactions. *Exp. Med. Surg.,* : 223-227.

BOYD, E.M., McLACHLAN, M.L., & PERRY, W.F. (1944) Experimental ammonia gas poisoning in rabbits and cats. *J. ind. Hyg. Toxicol.,* **26**: 29-34.

BRAGANGA, B.M., FAULKNER, P., & QUASTEL, J.M. (1953) Effects of inhibitors of glutamine synthesis in brain slices by ammonium ions. *Biochim. Biophys. Acta,* **10**: 83-88.

BREEDING, R.J., LODGE, J.P., Jr, PATE, J.B., SHEESLEY, D.C., KLONIS, H.B., FOGLE, B., ANDERSON, J.A., ENGLERT, T.R., HAAGENSON, P.L., MCBETH, R.B., MORRIS, A.L., POGUE, R., &

WARTBURG, A.F. (1973) Background trace concentrations in the central United States. *J. geophys. Res.,* **78**: 7057-7064.

BREEDING, R.J., KLONIS, H.B., LODGE, J.P., Jr, PATE, J.B., SHEESLEY, D.C., ENGLERT, T.R., & SEARS, D.R. (1976) Measurements of atmospheric pollutants in the St. Louis area. *Atmos. Environ.,* **10**: 181-184.

BRENNAN, E., LEONE, I.A., & DAINES, R.H. (1962) Ammonia injury to applies and peaches in storage. *Plant Dis. Rep.,* **46**: 792-795.

BRESSANI, R. & BRAHAM, J.E. (1977) Effect of frequency of feeding on blood serum urea changes in dogs. *J. Nutr. Rep. Int.,* **16**(3): 305-316.

BRETT, J.R. & ZALA, C.A. (1975) Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon *(Oncorhyncus nerka)* under controlled conditions. *J. Fish. Res. Board Can.,* **32**: 2479-2486.

BRETTHAUER, R. (1978) Some ecological limits of tolerance to *Ochromonas sociabilis. Verh. Internat. Verein. Limnol.,* **20**(3): 1850-1854.

BROCKWAY, D.R. (1950) Metabolic products and their effects. *Prog. Fish-Cult.,* **12**(3): 127-129.

BRODERIUS, S.J. & SMITH, L.L., Jr (1979) Lethal and sublethal effects of binary mixtures of cyanide and hexavalent chromium, zinc, or ammonia to the fathead minnow *Pimephales promelas* and rainbow trout *Salmo gairdneri. J. Fish. Res. Board Can.,* **36**(2): 164-172.

BRODERIUS, S.J., SMITH, L.L., Jr, & LIND, D.T. (1977) Relative toxicity of free cyanide and dissolved sulfide forms to the fathead minnow. *Pimephales promelas. J. Fish. Res. Board Can.,* **34**(12): 2323-2332.

BRODERIUS, S.J., DRUMMOND, R.A., FIANDT, J.T., & RUSSOM, C.L. (in press) Toxicity of ammonia to smallmouth bass, *Micropterus dolomieui,* as related to pH. *Environ. Toxicol. Chem..*

BRODERSON, J.R., LINDSEY, J.R., & CRAWFORD, J.E. (1976) The role of environmental ammonia in respiratory mycoplasmosis of rats. *Am. J. Pathol.,* **85**: 115-130.

BROSSET, C., ANDREASSON, K., & FERM, M. (1975) The nature and possible origin of acid particles observed at the Swedish west coast. *Atmos. Environ.,* **9**: 631-642.

BROWN, A.C. (1974) Observation on the effect of ammonium nitrate solutions on some common marine animals from Table Bay. *Trans. R. Soc. S. Afr.,* **41**(2): 217-223.

BROWN, V.M. (1968) The calculation of the acute toxicity of mixtures of poisons to rainbow trout. *Water Res.,* **2**(10): 723-733.

BROWN, R.H., DUDA, G.D., KORKES, S., & HANDLER, P. (1957) A colorimetric micromethod for determination of ammonia: the ammonia content of rat tissues and human plasma. *Arch. Biochem. Biophys.,* **66**: 301-309.

BROWN, V.M., JORDAN, D.H.M., & TILLER, B.A. (1969) The acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol and zinc. *J. Fish Biol.,* **1**(1): 1-9.

BUCKLEY, J.A. (1978) Acute toxicity of unionized ammonia to fingerling coho salmon. *Prog. Fish-Cult.,* **40**(1): 30-32.

BUCKLEY, J.A., WHITMORE, C.M., & LIMING, B.D. (1979) Effects of prolonged exposure to ammonia on the blood and liver glycogen of coho salmon *(Oncorhynchus kisutch). Comp. Biochem. Physiol.,* **63C**(2): 297-303.

BUIKEMA, A.I., Jr, CAIRNS, J., Jr, & SULLIVAN, G.W. (1974) Evaluation of *Philodina acuticornis* (Rotifera) as a bioassay organism for heavy metals. *Water Res. Bull.,* **10**(4): 648-661.

BUKHOVSKAYA, M.S., GINZBURG, S.L., & KHALIZOVA, O. (1966) *[Methods for determination of harmful substances in air. A practical guide,]* 2nd ed., Moscow, Meditaina (in Russian).

BULLIS, K.L., SNOEYENBOS, G.H., & VAN ROEKEL, H. (1950) A keratoconjunctivitis in chickens. *Poult. Sci.,* **29**: 386-389.

BURKHALTER, D.E. & KAYA, C.M. (1977) Effects of prolonged exposure to ammonia on fertilized eggs and sac fry of rainbow trout *(Salmo gairdneri). Trans. Am. Fish Soc.,* **106**(5): 470-475.

BURROWS, R.E. (1964) *Effects of acclimated excretory products on hatchery-reared salmonids,* Washington DC, Fish and Wildlife Service, US Department of the Interior, 12 pp (Research Report 66).

BUTTERWORTH, B. (1979) Nutritionally improved straw: the machines do the job. *Agric. Eng.,* **60**(7): 41-42.

BUYSMAN, E. (1984) *[Ammonia emissions in the Netherlands,]* Ministry of Housing, Physical Planning, and Environment, Directorate Air (Report No. 22) (in Dutch).

BUYSMAN, E., MAAS, J.F.M., & ASMAN, W.A.M. (1985) *Ammonia emission in Europe,* Utrecht, the Netherlands, Institute for Meteorology and Oceanography, State University Utrecht.

BYERRUM, R.U. & BENSON, A.A. (1975) Effect of ammonia on photosynthetic rate and photosynthate release by *Amphidinium carterae* (Dinophyceae). *J. Phycol.,* **11**: 449-452.

CALABRESE, E.J. (1978) *Pollutants and high risk groups,* New York, Wiley-Interscience Publications.

CALAMARI, D., MARCHETTI, R., & VAILATI, G. (1977) [Effect of prolonged treatments with ammonia on stages of development of *Salmo gairdneri.] Nuovi Ann. Ig. Microbiol.*, **28**(5): 333-345. (in Italian).

CALAMARI, D., MARCHETTI, R., & VAILATI, G. (1981) Effects of long-term exposure to ammonia on the developmental stages of rainbow trout (*Salmo gairdnerii* Richardson). *Rapp. P.-V. Réun. Cons. Int. Explor. Mer,* **178**: 81-86.

CAMERON, J.N. (1979) Excretion of CO_2 in water-breathing animals. *Marine Biol. Lett.*, **1**: 3-13.

CAMERON, J.N. & HEISLER, N. (1983) Studies of ammonia in the rainbow trout physio-chemical parameter, acid-base behavior, and respiratory clearance. *J. exp. Biol.,* **105**:: 107-125.

CAMERON, G.R. & SHEIKH, A.H. (1951) Experimental production of pulmonary edema with ammonium salts, together with classification of lung oedemas. *J. Pathol. Bacteriol.,* **63**: 609-617.

CAMPBELL, C.L., DAWES, R.K., DEOLALKAR, S., & MERRITT, M.C. (1958) Effect of certain chemicals in water on the flavor of brewed coffee. *Food Res.,* **23**: 575-579.

CAPLIN, M. (1941) Ammonia gas poisoning. Forty-seven cases in a London shelter. *Lancet,* **2**: 95-96.

CAPUCO, A.V. (1977) *Ammonia, a modulator of 3T3 cell growth,* Ithaca, New York, Cornell University (Ph.D Thesis).

CARPENTER, C.P., SMYTH, H.S., Jr, & SHAFFER, C.B. (1948) The acute toxicity of ethylene imine to small animals. *J. ind. Hyg. Toxicol.,* **30**: 2-6.

CARY, G.A. (1976) *A report on the assessment of aquatic environmental impact of Union Carbide's Uravan operations: on-site toxicity bioassays,* Tarrytown, New York, Aquatic Environmental Sciences, Union Carbide Corporation, 67 pp (Report prepared for Metals Division, Union Carbide Corporation, Uravan, Colorado).

CASTELL, D.O. & MOORE, E.W. (1971) Ammonia absorption from the human colon. *Gastroenterology,* **60**(1): 33-42.

CATEDRAL, F.F., COLOSO, R., VALERA, N., CASALMIR, C.M., & QUIBUYEN, A.T. (1977a) Effect of some physico-chemical factors on the survival and growth of *Penaeus monodon* postlarvae. *Q. Res. Rep. Aquacult. Dep. Southeast Asian Fish. Dev. Cent.,* **1**(3): 13-16.

CATEDRAL, F.F., GEROCHI, D.D., QUIBUYEN, A.T., & CASALMIR, C.M. (1977b) Effect of nitrite, ammonia, and temperature on *P. monodon* larvae. *Q. Res. Rep. Aquacult. Dep. Southeast Asian Fish. Dev. Cent.,* **1**(3): 9-12.

CHAMP, M.A., LOCK, J.T., BJORK, C.D., KLUSSMANN, W.G., & MCCULLOUGH, J.D., Jr (1973) Effects of anhydrous ammonia on a central Texas pond and a review of previous research with ammonia in fisheries management. *Trans. Am. Fish. Soc.,* **102**(1): 73-82.

CHARLES, D.R. & PAYNE, C.G. (1966a) The influence of graded levels of atmospheric ammonia on chickens. I. Effects on respiration and on the performance of broilers and replacement growing stock. *Br. Poult. Sci.,* **7**: 177-187.

CHARLES, D.R. & PAYNE, C.G. (1966b) The influence of graded levels of atmospheric ammonia on chickens. II. Effects on the performance of laying hens. *Br. Poult. Sci.,* **7**: 189-198.

CHEMICAL AND ENGINEERING NEWS (1980) C and EN's top fifty chemical products. *Am. Chem. Soc.,* : 16.

CHIPMAN, W.A., Jr (1934) *The role of pH in determining the toxicity of ammonium compounds,* Columbia, Missouri, University

of Missouri, 153 pp (Ph.D. Thesis).

CLAIBORNE, J.B., EVANS, D.H., & GOLDSTEIN, L. (1982) Fish branchial Na^+-K^+-activated ATPase. *J. exp. Biol.,* **96**: 431-434.

CLAIBORNE, J.B. & HEISLER, N. (1984) Acid-base regulation in the carp *(Cyprinus carpio)* during and after exposure to environmental hypercapnia. *J. exp. Biol.,* **108**: 25-43.

CLAWSON, A.J. & ARMSTRONG, W.D. (1981) Ammonium polyphosphate as a source of phosphorous and nonprotein nitrogen for monogastrics (in corn-soybean meal diets for growing rats and growing finishing [G-F] pigs). *J. anim. Sci.,* **52**(1): 1-7.

CLOSE, L.G., CATLIN, F.I., & COHN, A.M. (1980) Acute and chronic effects of ammonia burns of the respiratory tract. *Arch. Otolaryngol.,* **106**: 151-158.

COLE, T.J., COTES, J.E., JOHNSON, G.R., & MARTIN, H. (1977) Ventilation cardiac frequency and pattern of breathing during exercise in men exposed to *o*-chlorobenzylidene malonitril and ammonia gas in low concentrations. *Q. J. exp. Physiol.,* **62**: 341-351.

COLT, J.E. (1978) *The effects of ammonia on the growth of channel catfish, Ictalurus punctatus,* Davis, California, University of California, 185 pp (Ph.D. Thesis).

COLT, J.E. & TCHOBANOGLOUS, G. (1976) Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish, *Ictalurus punctatus. Aquaculture,* **8**(3): 209-224.

COLT, J.E. & TCHOBANOGLOUS, G. (1978) Chronic exposure of channel catfish, *Ictalurus punctatus* to ammonia: effects on growth and survival. *Aquaculture,* **15**(4): 353-372.

CONN, H.O. (1972) Studies of the source and significance of blood ammonia. IV. Early ammonia peaks after ingestion of ammonium salts. *Yale J. Biol. Med.,* **45**: 543-549.

CONSTANTINE, D.G. (1958) Bleaching of hair pigment in bats by the atmosphere in caves. *J. Mammal,* **39**: 513-520.

COOMBE, J.B., TRIBE, D.E., & MORRISON, J.W. (1960) Some experimental observations on the toxicity of urea to sheep. *Aust. J. agric. Res.,* **11**: 247-256.

COON, R.A., JONES, R.A., JENKINS, L.J., Jr, & SIEGEL, J. (1970) Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. *Toxicol. appl. Pharmacol.,* **16**: 646-655.

COTTERILL, O.J. & NORDSKOG, A.W. (1954) Influence of ammonia on egg white quality. *Poult. Sci.,* **33**: 432-434.

CRIBB, G.S. (1964) Nitrogen. In: *Mellor's comprehensive treatise on inorganic and theoretical chemistry,* London, Longmans, Vol. 8, Suppl. I, pp. 276-327.

CURTIS, S.E. (1972) Air environment and animal performance. *J. anim. Sci.,* **35**: 628-634.

DALHAMN, T. (1963) Effect of ammonia alone and combined with

carbon particles on ciliary activity in the rabbit trachea *in vivo,* with studies of the absorption capacity of the nasal cavity. *Int. J. Air Water Pollut.,* **7**: 531-539.

DALHAMN, T. & SJOHOLM, J. (1961) Studies of SO_2, NO_2, and NH_3: effect on ciliary activity in the rabbit trachea of single *in vitro* exposure and resorption in rabbit nasal cavity. *Acta physiol. Scand.,* **58**: 287-291.

DALLAVALLE, J.M. & DUDLEY, H.C. (1939) Evaluation of odor nuisance in the manufacture of kraft paper. *Public Health Rep.*, **54**(1): 35-43.

DANECKER, E. (1964) [The jauche poisoning of fish: an ammonia poisoning.] *Osterr. Fischerei.,* **3/4**: 55-68. (in German).

DAVIES, B.M.A. & YUDKIN, J. (1952) Studies in biochemical adaptation. The origin of urinary ammonia as indicated by the effect of chronic acidosis and alkalosis on some renal enzymes in the rat. *Biochem. J.,* **52**: 407-412.

DAY, D.L., HANSEN, E.L., & ANDERSON, S. (1965) Gases and odors in confinement swine buildings. *Trans. Am. Soc. Agric. Eng.,* **8**: 118-121.

DEAL, P.H., SOUZA, K.A., & MACK, H.M. (1975) High pH, ammonia toxicity, and the search for life on the Jovian planets. *Origins Life,* **6**(4): 561-573.

DEAN, J.A., ed. (1979) *Lange's handbook of chemistry,* 12th ed., New York, McGraw-Hill Book Company.

DEGRAEVE, G.M., OVERCAST, R.L., & BERGMAN, H.L. (1980) Toxicity of underground coal gasification condenser water and selected constitutents to aquatic biota. *Arch. environ. Contam. Toxicol.,* **9**(5): 543-555.

DELISTRATY, D.A., CARLBERG, J.M., VAN OLST, J.C., & FORD, R.F. (1977) Ammonia toxicity in cultured larvae of the American lobster *(Homarus americanus).* In: *Proceedings of the 8th Annual Meeting of the World Mariculture Society, San Jose, Costa Rica,* pp. 647-673.

DEMEREC, M., BERTANI, G., & FLINT, J. (1951) A survey of chemicals for mutagenic action on *E. coli. Am. Nat.,* **85**: 119.

DEMUYNCK, M., RAHN, K.A., JANSSENS, M., & DAMS, R. (1976) Chemical analysis of airborne particulate matter during a period of unusually high pollution. *Atmos. Environ.,* **10**: 21-26.

DE VOOYS, G.G.N. (1968) Formation and excretion of ammonia in Teleostei. I. Excretion of ammonia through the gills. *Arch. int. Physiol. Biochimie.,* **76**: 268-272.

DODD, K.T. & GROSS, D.R. (1980) Ammonia inhalation toxicity in cats. *Arch. environ. Health,* **35**: 6-14.

DOIG, P.A. & WILLOUGHBY, R.A. (1971) Response of swine to atmospheric ammonia and organic dust. *J. Am. Vet. Med. Assoc.,* **159**: 1353-1361.

DOWDEN, B.F. & BENNETT, H.J. (1965) Toxicity of selected chemicals to certain animals. *J. Water Pollut. Control. Fed.,*

37(9): 1308-1316.

DOWNING, K.M. & MERKENS, J.C. (1955) The influence of dissolved-oxygen concentration on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdneri* Richardson). *Ann. appl. Biol.,* **43**(2): 243-246.

DREWES, D.R. & HALES, J.M. (1980) *Removal of pollutants from power plant plumes by precipitation,* Richland, Washington, Battelle-Northwest, US Electric Power Research Institute (Report to the EPRI).

DRIEDZIC, W.R. & HOCHACHKA, P.W. (1976) Control of energy metabolism in fish white muscle. *Am. J. Physiol.,* **230**: 579-582.

DRUMMOND, J.G., CURTIS, S.E., SIMON, J., & NORTON, H.W. (1980) Effects of aerial ammonia on growth and health of young pigs. *J. anim. Sci.,* **50**: 1085-1091.

DUDA, G.D. & HANDLER, P. (1958) Kinetics of ammonia metabolism *in vivo. J. biol. Chem.,* **232**: 303-314.

DU RUISSEAU, J.P., GREENSTEIN, J.P., WINITZ, M., & BIRNBAUM, S.M. (1957) Studies on the metabolism of amino acids and related compounds *in vivo.* VI. Free amino acid levels in the tissues of rats protected against ammonia toxicity. *Arch. Biochem. Biophys.,* **68**: 161-171.

EADS, R.B., WISEMAN, J.S., GRIMES, J.E., & MENZIES, G.C. (1955) Wildlife rabies in Texas. *Public Health Rep.,* **70**: 995-1000.

ECKERT, J.W. (1967) Application and use of postharvest fungicides. In: Torgeson, D.C., ed. *Fungicides,* New York, Academic Press, pp. 341.

ECKERT, J.W., KOLBEZEN, M.J., & GARBER, M.J. (1963) Evaluation of ammonia-generating formations for control of citrus fruit decay. *Phytopathology,* **53**: 140-148.

ECOLOGICAL ANALYSTS, INC. (1981) *The sources, chemistry, fate, and effects of ammonia in aquatic environments,* Washington DC, American Petroleum Institute.

EDELMANN, C.M., Jr, BIOCHIS, H., SORIANO, J.R., & STARK, H. (1967) The renal response of children to acute ammonium chloride acidosis. *Pediat. Res.,* **1**: 452-460.

EDJTEHADI, M., SZABUNIEWICZ, M., & EMMANUEL, B. (1978) Acute urea toxicity in sheep. *Can. J. Comp. Med.,* **42**: 63-68.

EDWARDS, J.G. & CONDORELLI, L. (1928) Studies on aglomerular and glomerular kidneys. II. Physiological. *Am.J. Physiol.,* **86**: 383-398.

EGGELTON, A.E.J. (1969) The chemical composition of atmospheric aerosols on Tees-side and its relation to visibility. *Atmos. Environ.,* **3**: 355-372.

EGLE, J.L., Jr (1973) Retention of inhaled acetone and ammonia in the dog. *Am. Ind. Hyg. Assoc. J.,* **34**(12): 533-539.

ELKINS, H.B. (1950) *The chemistry of industrial toxicology,* New York, John Wiley and Sons, 84 pp.

ELKINS, H.B. (1959) Ammonia, NH_3. In: *The chemistry of industrial toxicology,* 2nd ed., New York, John Wiley and Sons, 86 pp.

EMERSON, K., RUSSO, R.C., LUND, R.E., & THURSTON, R.V. (1975) Aqueous ammonia equilibrium calculations: effect of pH and temperature. *J. Fish. Res. Board Can.,* **32**: 2379-2383.

EMERY, R.M. & WELCH, E.B. (1969) *The toxicity of alkaline solutions of ammonia to juvenile bluegill sunfish (Lepomis macrochirus* Raf.), Chattanooga, Tennessee, Water Quality Branch, Division of Health and Safety, Tennessee Valley Authority, 31 pp (Mimeo).

EPEL, D., STEINHARDT, R., HUMPHREYS, T., & MAZIA, D. (1974) An analysis of the partial metabolic derepression of sea urchin eggs by ammonia; the existence of independent pathways. *Dev. Biol.,* **40**: 245-255.

EPIFANO, C.E. & SRNA, R.F. (1975) Toxicity of ammonia, nitrite ion, nitrate ion, and orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica. Mar. Biol.,* **33**(3): 241-246.

ERNST, R.A. (1968) The effect of ammonia on poultry. *Feedstuffs,* **40**(32): 40.

EUROPEAN INLAND FISHERIES ADVISORY COMMISSION (1970) *Water quality criteria for European freshwater fish. Report on ammonia and inland fisheries,* pp. 12 (EIFAC Technical Paper No. 11) (Also in: *Water Res.,* **7**(7): 1011-1022 (1973)).

EVANS, D.H. (1977) Further evidence for Na/NH_4 exchange in marine teleost fish. *J. exp. Biol.,* **70**: 213-220.

EVANS, D.H. (1980) Osmotic and ionic regulation by freshwater and marine fishes. In: Ali, M.A. ed. *Environmental Physiology of Fishes,* New York, Plenum Publishing Co., pp. 93-122.

EVANS, D.H., KORMANIK, G.A., & KRASNY, E.J. (1979) Mechanisms of ammonia and acid extrusion by the little skate, *Raja erinacea. J. exp. Zool.,* **208**: 431-437.

EVANS, J.W. (1979) *The construction and use of a continuous-flow bioassay apparatus to determine a preliminary un-ionized ammonia 96-h* LC_{50} *for the crayfish, Orconectes nais,* Lawrence, Kansas, University of Kansas, 76 pp (M.S. Thesis).

FARBER, R.J. & ROSSANO, A.T. (1975) A new approach for measuring atmospheric concentrations of ammonia. *Paper presented at Annual Meeting of the Pacific Northwest International Section of the Air Pollution Control Association, Vancouver, British Columbia, November 19-21,* 36 pp.

FASEB (1974) *Evaluation of the health aspects of certain ammonium salts as food ingredients,* Springfield, Virginia, Federation of American Societies for Experimental Biology, 21 pp (Prepared for the US Food and Drug Administration under Contract No. FDA 72-85) (US NTIS PPB-254-532).

FAUCONNEAU, B. & LUQUET, P. (1979) Influence d'une élévation de température sur l'évolution de l'aminoacidémie et de l'ammoniémie après le repas chez la truite arc en ciel *(Salmo gairdneri* R.). *Ann. Biol. anim. Biochim. Biophys.,* **19**(4A): 1063-1079.

FAZEKAS, I.G. (1939) Experimental suprarenal hypertrophy induced by ammonium hydroxide. *Endokrinologie,* **21**: 315-337.

FAZEKAS, I.G. (1949) [Experimental data on influencing ovarian functions by simple compounds.] *Orv. Hetil.,* **90**: 777-781 (in German).

FAZEKAS, I.G. (1954a) [Enlargement of the parathyroids by treatment with simple acidotic compounds.] *Kirchow's Arch. Pathol. Anat. Physiol.,* **324**: 531-542 (in German).

FAZEKAS, J.F., TICKTIN, H.E., EHRMENTRAUT, W.R., & ALMAN, R.W. (1956) Cerebral metabolism in hepatic insufficiency. *Am. J. Med.,* **21**: 843-849.

FEPCC (1972) [Investigation of air pollution in the city of Tsuruga.] *Annu. Rep. Fukui Environ. Pollut. Control Centre,* **1**: 88-116 (in Japanese).

FERGUSON, W.S., KOCH, W.C., WEBSTER, L.B., & GOULD, J.R. (1977) Human physiological response and adaptation to ammonia. *J. occup. Med.,* **19**: 319-326.

FETH, J.H. (1966) Nitrogen compounds in natural water. A review. *Water Resour. Res.,* **2**: 41-58.

FISHER, D.W., GAMBELL, A.W., LIKENS, G.E., & BURMANN, F.H. (1968) Atmospheric contributions to water quality of streams in the Hubbard Brook Experimental Forest, New Hampshire. *Water Resour. Res.,* **4**: 1115-1126.

FLAKS, A. & CLAYSON, D.B. (1975) The influence of ammonium chloride on the induction of bladder tumors by 4-ethyl-sulfonylnaphthalene-1-sulfonamide. *Br. J. Cancer,* **31**: 585.

FLAKS, A., HAMILTON, J.M., & CLAYSON, D.B. (1973) Effect of ammonium chloride on incidence of bladder tumours induced by 4-ethylsulfonylnapthalene-1-sulfonamide. *J. Natl Cancer Inst.,* **51**: 2007.

FLORKIN, M. & DUCHATEAU, G. (1943) Les formes du système enzymatique de l'uricalyse et l'évolution du catabolisme purique chez les animaux. *Arch. int. Physiol.,* **53**: 267-307.

FLURY, K.E., DINES, D.E., RODARTE, J.R., & RODGERS, R. (1983) Airway obstruction due to inhalation of ammonia. *Mayo Clin. Proc.,* **58**: 389-393.

FLYNN, R.J. (1968) A new cage cover as an aid to laboratory rodent disease control. *Proc. Soc. Exp. Biol. Med.,* **129**: 714-717.

FORSTER, R.P. & GOLDSTEIN, L. (1969) Formation of excretory products. In: Hoar, W.S. & Randall, D.J. ed. *Fish Physiology,* New York, Academic Press, Vol. 1, pp. 313-350.

FOSTER, G.L., SCHOENHEIMER, R., & RITTENBERG, D. (1939) Studies in protein metabolism. V. The utilization of ammonia

for amino acid and creatine formation in animals. *J. biol. Chem.,* **127**: 319-327.

FRANK, J.F. (1948) The toxicity of sodium chlorate herbicides. *Can. J. comp. Med.,* **12**: 216-218.

FRASER, D.I., DYER, W.J., WEINSTEIN, H.M., DINGLE, J.R., & HINES, J.A. (1966) Glycolytic metabolites and their distribution at death in the white and red muscle of cod following various degrees of antemortem muscular activity. *Can. J. Biochem.,* **44**: 1015-1033.

FREEDMAN, L.R. & BEESON, P.B. (1961) Experimental pyelonephritis. VIII. The effect of acidifying agents on susceptibility to infection. *Yale J. Biol. Med.,* **33**: 318.

FRIZZOLA, J.A. & BAIER, J.H. (1975) Contaminants in rainwater and their relation to water quality. *Water Sewage Works,* **122**: 94-95.

FROMM, P.O. (1963) Studies on renal and extra-renal excretion in a freshwater teleost, *Salmo gairdneri. Comp. Biochem. Physiol.*, **10**: 121-128.

FROMM, P.O. (1970) Effect of ammonia on trout and goldfish. In: *Toxic action of water soluble pollutants on freshwater fish,* Washington DC, US Environmental Protection Agency, pp. 9-22 (EPA Water Pollution Control Research Series No. 18050 DST 12/70).

FROMM, P.O. & GILLETTE, J.R. (1968) Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout *(Salmo gairdneri). Comp. Biochem. Physiol.,* **26**: 887-896.

FUHRER, J. (1985) Formation of secondary airpollutants and their occurrence in Europe. *Experientia (Basel),* **41**: 286-301.

FURST, P., JOSEPHSON, B., MASCHIO, G., & VINNARS, E. (1969) Nitrogen balance after intravenous and oral administration of ammonium salts to man. *J. appl. Physiol.,* **26**(1): 13-22.

FURST, P., JONSSON, A., JOSEPHSON, B., & VINNARS, E. (1970) Distribution in muscle and liver vein protein of ^{15}N administered as ammonium acidosis to man. *J. appl. Physiol.,* **29**(3): 307-312.

GALL, W.K. (1980) *Effect of cow faeces on the ecology of laboratory streams,* Madison, Wisconsin, University of Wisconsin, pp. 73-83 (M.S. Thesis).

GAY, W.M.B., CRANE, C.W., & STONE, W.D. (1969) The metabolism of ammonia in liver disease: a comparison of urinary data following oral and intravenous loading of [^{15}N] ammonium lactate. *Clin. Sci.,* **37**: 815-823.

GEORGII, H.W. (1963) Oxides of nitrogen and ammonia in the atmosphere. *J. geophys. Res.,* **68**: 963-970.

GEORGII, H.W. & MULLER, W.J. (1974) On the distribution of ammonia in the middle and lower trophosphere. *Tellus,* **26**: 180-184.

GIBBS, M. & CALO, N. (1959) Factors affecting light induced fixations of carbon dioxide by isolated spinach chloroplasts.

Plant Physiol., **34**: 318-323.

GIBSON, G.E., ZIMBER, A., KROOK, L., & VISEK, W.J. (1971) Nucleic acids and brain and intestinal lesions in ammonia intoxicated mice. *Fed. Proc.,* **30**: 578.

GIGUZ, T.L. (1968) Effect of low concentrations of ammonia and nitrogen oxides on adolescents undergoing vocational training in the chemical industry. *Hyg. Sanit.,* **33**(7-9): 431-434.

GILLETTE, L.A., MILLER, D.L., & REDMAN, H.E. (1952) Appraisal of a chemical waste problem by fish toxicity tests. *Sewage ind. Wastes,* **24**(11): 1397-1401.

GILLIAM, J.W., DANIELS, R.B., & LUTZ, J.F. (1974) Nitrogen content of shallow ground water in North Carolina Coastal Plain. *J. environ. Qual.,* **3**: 147-151.

GIRARD, J.O. & PAYAN, P. (1980) Ion exchanges through respiratory and chloride cells in freshwater and seawater adapted teleosteans. *Am. J. Physiol.,* **238**: R260-R268 (Regulatory Integrative Comp. Physiol. 7).

GOLDHABER, M.B. & KAPLAN, I.R. (1974) The sulfur cycle. In: Goldberg, D., ed. *The sea: ideas and observations on progress in the study of the seas,* New York, John Wiley and Sons, Vol. 5, pp. 569-655.

GOLDMAN, A.S. & YAKOVAC, W.C. (1964) Salicylate intoxication and congenital anomalies. *Arch. environ. Health,* **8**: 648-656.

GOLDSTEIN, L., CLAIRBORNE, J.B., & EVANS, D.E. (1982) Ammonia excretion by the gills of two marine teleost fish: the importance of NH_4^+ permeance. *J. exp. Zool.,* **219**: 395-397.

GOLDSTEIN, L. & FORSTER, R.P. (1961) Source of ammonia excreted by the gills of the marine teleost, *Myoxocephalus scropius. Am. J. Physiol.,* **200**(5): 1116-1118.

GOLYAKOVA, L.P. (1971) [Present-day state of industrial hygiene problems in hydrometallurgical production of tungsten and molybdenum salts and oxides.] *Gig. Tr. Prof. Zabol.,* **15**(2): 4-8 (in Russian with English summary).

GOODMAN, L.S. & GILMAN, A. (1970) *The pharmacological basis of therapeutics,* 4th ed., New York, The Macmillan Company, 1794 pp.

GORDON, R.J. & BRYAN, R. (1973) Ammonium nitrate in airborne particles in Los Angeles. *Environ. Sci. Technol.,* **7**: 645-647.

GORDON, H.H., MCNAMARA, H., & BENJAMIN, H.R. (1948) Response of young infants to ingestion. *Pediatrics,* **2**: 290-302.

GRANT, W.M. (1974) *Toxicology of the eye: drugs, chemicals, plant venoms,* 2nd ed., Springfield, Illinois, C.C. Thomas, pp. 96-101, 121-128.

GREENSTEIN, J.P., WINITZ, M., GULLINO, P., BIRNBAUM, S.M., & OTEY, M.C. (1956) Studies on the metabolism of amino acids and related compounds *in vivo.* III. Prevention of ammonia toxicity by arginine and related compounds. *Arch. Biochem.*

Biophys., **64**: 342-354.

GREGORY, R.B. (1977) Synthesis and total excretion of waste nitrogen by fish of the *Periopthalmus* (mudskipper) and *Scartelass* families. *Comp. Biochem. Physiol.,* **51A**: 33-36.

GRINDLEY, J. (1946) Toxicity to rainbow trout and minnows of some substances known to be present in waste water discharged to rivers. *Ann. appl. Biol.,* **33**(1): 103-112.

GROLLMAN, A. (1929) The urine of the goosefish *(Lophius piscatorius):* its nitrogenous constituents with special reference to the presence in it of trimethylamine oxide. *J. Biol. Chem.,* **81**: 267-278.

GROSJEAN, D., DOYLE, G.J., MISCHKE, T.M., POE, M.P., FITZ, D.R., SMITH, J.P., & PITTS, J.N., Jr (1976) The concentration, size distribution, and modes of formation of particulate nitrate, sulfate, and ammonium compounds in the eastern part of the Los Angeles Basin. Presented at: *The 69th Annual Meeting of the Air Pollution Control Association, Portland, Oregon, June 27 - July 1 1976,* 14 pp (Paper 76-20.3).

GROSSI, C.E., PRYTZ, B., & ROUSSELOT, L.M. (1968) Adenosine triphosphate in prevention of ammonia intoxication in dogs. *Surg. Forum,* **19**: 363-369.

GRUBE, H.J. (1973) [Load and loading capacity of submerged macrophytes in running water in southern Lower Saxony.] *Verh. Ges. Okol., Saarbrücken:* 103-109 (in German with English summary).

GUERIN-ANCEY, O. (1976a) Etude experimentale de l'excretion azotee du bar *(Dicentrarchus labrax)* en cours de croissance. II. Effets du jeune sur l'excretion d'ammoniac et d'uree. *Aquaculture,* **9**(2): 187-294.

GUERIN-ANCEY, O. (1976b) Etude experimentale de l'excretion azotee du bar *(Dicentrarchus labrax)* en cours de croissance. III. Effets du volume et de la concentration initiale en ammoniac sur l'excretion d'ammoniac et d'uree. *Aquaculture,* **9**(2): 253-258.

GUPTA, B.N., KHANNA, R.N., & DATTA, K.K. (1979) Toxicological studies of ammonium sulfamate in rat after repeated oral administration. *Toxicology,* **13**(1): 45-49.

GYORE, K. & OLAH, J. (1980) Ammonia tolerance of *Moina rectirostris* Leydig (Cladocera). *Aquaculture Hungarica (Szarvas),* **2**: 50-54.

HAGGARD, H.W. (1924) Action of irritant gases upon the respiratory tract. *J. ind. Hyg.,* **5**: 390-398.

HAHN, M., MASSEN, O., NENCKI, N., & PAWLOW, J. (1983) [The connection between the vena cava inferior and the portal vein and its consequences for the organism.] *Arch. Exp. Path. Pharmakol.,* **32**: 161-210 (in German).

HALL, L.W., Jr, BUIKEMA, A.L., Jr, & CAIRNS, J., Jr (1978) The effects of a stimulated refinery effluent on the grass shrimp, *Palaemonetes pugio. Arch. environ. Contam. Toxicol.,* **7**(1) 23-35.

HANTZSCH, S. & LAHMANN, E. (1970) [Ammonia determinations in city air.] *Schriftner. Ver. Wasser- Boden- Lufthyg. (Berlin-Dahlem),* **33**: 35-39 (in German).

HARADER, R.R. & ALLEN, G.H. (1983) Ammonia toxicity to chinook salmon parr: reduction in saline water. *Trans Am. Fish. Soc.,* **112**: 834-837.

HARDING, A.J. (1959) *Ammonia manufacture and uses,* London, Oxford University Press.

HAYNES, R.J. & GOH, K.M. (1977) Evaluation of potting media for commercial nursery production of container grown plants. II. Effects of media, fertilizer nitrogen and nitrification inhibitor on yield and nitrogen uptake of *Callistephus chinensis* (L.) nees "Pink Princess". *N.Z. J. Agric. Res.,* **20**: 371-381.

HAWKINS, R.A., MILLER, A.L., NIELSEN, R.C., & VEECH, R.L. (1973) The acute action of ammonia on rat brain metabolism *in vivo. Biochem. J.,* **134**: 1001-1008.

HAWKINS, D.B., DEMETER, M.J., & BARNETT, T.E. (1980) Caustic ingestion: Controversies in management. A review of 14 cases. *Laryngoscope,* **90**: 98-109.

HAZEL, C.R., THOMSEN, W., & MEITH, S.J. (1971) Sensitivity of striped bass and stickleback to ammonia in relation to temperature and salinity. *Calif. Fish Game,* **57**(3): 138-153.

HAZEL, R.H., BURKHEAD, C.E., & HUGGINS, D.G. (1979) *The development of water quality criteria for ammonia and total residual chlorine for the protection of aquatic life in two Johnson County, Kansas streams,* Washington DC, Office of Water Research and Technology, US Department of the Interior, 146 pp.

HEALY, T.V. (1974) Ammonia and related pollutants at Harwell. *Atmos. Environ.,* **8**: 81-83.

HEALY, T.V., MCKAY, H.A.C., PILBEAM, A., & SCARGILL, D. (1970) Ammonia and ammonium sulfate in the troposphere over the United Kingdom. *J. geophys. Res.,* **75**: 2317-2321.

HEIL, G.W. & DIEMONT, W.M. (1983) Raised nutrient levels change heathland into grassland. *Vegetatio,* **53**: 113-120.

HEISLER, N. (1984) Acid-base regulation in fishes. In: Hoar, W. & Randall, D.J., ed. *Fish physiology,* New York, Academic Press, Vol. 10A.

HELMERS, S., TOP, F.H., & KNAPP, L.W., Jr (1971) Ammonia injuries in agriculture. *J. Iowa Med. Soc.,* **61**: 271-280.

HEMENS, J. (1966) The toxicity of ammonia solutions to the mosquito fish (*Gambusia affinis* Baird & Girard). *J. Proc. Inst. Sewage Purif.,* **65**: 265-271.

HENIS, Y. & CHET, I. (1967) Mode of action of ammonia on *Sclerotium rolfsii. Phytopathology,* **57**: 425-427.

HERBERT, D.W.M. (1956) La toxicité d'un effluent d'eau d'égout. *Bull. Cent. belge Etude Doc. Eaux,* **32**: 115-120.

HERBERT, D.W.M. (1962) The toxicity of rainbow trout of

spent still liquors from the distillation of coal. *Ann. appl. Biol.,* **50**(4): 755-777.

HERBERT, D.W.M. & SHURBEN, D.S. (1965) The susceptibility of salmonid fish to poisons under estuarine conditions. II. Ammonium chloride. *Int. J. Air Water Pollut.,* **9**: 89-91.

HIDY, G.M. (1974) *Characterization of aerosols in California (ACHEX),* Thousand Oaks, California, Rockwell International, Vol. 1-4 (Final Report prepared for California Air Resources Board).

HILLABY, B.A. & RANDALL, D.J. (1979) Acute ammonia toxicity and ammonia excretion in rainbow trout *(Salmo gairdneri). J. Fish. Res. Board Can.,* **36**(6): 621-629.

HINTZ, H.F., LOWE, J.E., CLIFFORD, A.J. ET AL. (1970) Ammonia intoxication resulting from urea ingestion by ponies. *J. Am. Vet. Med. Assoc.,* **157**: 963-966.

HOEFT, R.G., KENNEY, D.R., & WALSH, L.M. (1972) Nitrogen and sulfur in precipitation and sulfur dioxide in the atmosphere in Wisconsin. *J. environ. Qual.,* **1**: 203-208.

HOGAN, J.P. (1961) The absorption of ammonia through the rumen of sheep. *Aust. J. biol. Sci.,* **14**: 448-460.

HOLETON, G.F., NEUMANN, P., & HEISLER, N. (1983) Branchial ion exchange and acid-base regulation after strenous exercise in rainbow trout. *Resp. Physiol.,* **51**: 303-318.

HOLLAND, G.A., LASATER, J.E., NEUMANN, E.D., & ELDRIDGE, W.E. (1960) *Experiments with inorganic pollutants - ammonia. Toxic effects of organic and inorganic pollutants on young salmon and trout,* State of Washington, Department of Fisheries, pp. 183-187 (Research Bulletin No. 5).

HOLT, G.J. & ARNOLD, C.R. (1983) Effects of ammonia and nitrite on growth and survival of red drum eggs and larvae. *Trans. Am. Fish. Soc.,* **112**: 314-318.

HOLT, J.D. & MALCOLM, S.A. (1979) Evaluation of fish loading rates in regulatory static bioassays. *Tech. Rep. -Fish. Mar. Serv. (Can.).,* **862**: 146-160.

HORNE, F.R. & MCINTOSH, S. (1979) Factors influencing distribution of mussels in the Blanco River of central Texas. *Nautilus,* **94**(4): 119-133.

HSIA, Y.E. (1974) Inherited hyperammonemic syndromes. *Gastroenterology,* **67**: 347-374.

HUNGER, W. (1978) [Dying off of old spruce trees in the vicinity of a pig fattening station.] *Beitrage Forstwirtschaft,* **4**: 188-189 (in German).

HUNT, R.D. (1977) Measurement of partial pressure of ammonia in breath and arterial blood. Evidence for secretion of ammonia into expired gas by metabolism of monoamines in the lung. *Diss. Abstr. Int. Ser. B,* **37**: 3279.

ILO (1970) Permissible levels of toxic substances in the working environment. In: *Proceedings of the 6th Session of the Joint ILO/WHO Committee on Occupational Health, Geneva, 4-10*

June 1968, Geneva, International Labour Office, 405 pp.

INDUSTRIAL BIO-TEST LABORATORIES, INC. (1973) *Irritation threshold evaluation study with ammonia,* Industrial Bio-Test Laboratories, Inc. (Report to International Institute of Ammonia Refrigeration, Publication No. 663-03161).

IWAOKA, W.T., OKRONE, C.A., SULLIVAN, J.J., & JOHNSON, C.A. (1981a) Effect of pH and ammonium ions on mutagenic activity in cooked beef. *Cancer Lett.,* **12**: 335.

IWAOKA, W.T., URONE, C.A., SULLIVAN, J.J., MEAKER, E.H., & JOHNSON, C.A. (1981b) A source of error in mutagen testing of foods. *Cancer Lett.,* **11**: 225.

JACOBS, M.H. (1940) Some aspects of cell permeability to weak electrolytes. *Cold Spring Harbour Sym. Quant. Biol.,* **8**: 30-39.

JACQUEZ, J.A., POPPELL, J.W., LAWRENCE, W., Jr, & ROBERTS, K.E. (1957) Clinical significance of the partial pressure of ammonia (pNH_3) in patients with ammonium toxicity. *Clin. Res. Proc.,* **5**: 20 (Abstract).

JACQUEZ, J.A., POPPELL, J.W., & JELTSCH, R. (1959) Partial pressure of ammonia in aveolar air. *Science,* **129**: 269-270.

JAFFE, H.L., BODANSKY, A., & BLAIR, J.E. (1932a) Effects of parathormone (parathyroid preparation) and ammonium chloride on bones of rabbits. *J. exp. Med.,* **55**: 695-701.

JAFFE, H.L., BODANSKY, A., & CHANDLER, J.P. (1932b) Ammonium chloride decalcification, as modified by calcium intake: the relation between generalized osteoporosis and osteitis fibrosa. *J. exp. Med., 56: 823-834.*

JANICKI, R.H. (1970) Renal adaptation during chronic NH_4Cl acidosis in the rat: no role for hyperplasia. Am. J. Physiol., **219**(3): 613-618.

JANSSEN, Th. W. (1982) *[Intensive animal husbandry in relation to space and environment,]* Utrecht, The Netherlands, Dutch State Forest Service (in Dutch).

JOHANNING, G.L., MUHRER, M.E., & GADDY, J.H. (1978) Nutritional evaluation of chlorinated-ammoniated corn stover. Paper presented at: *The Joint Meeting of the American Dairy Science Association and the American Society of Animal Science, 11 July 1978.*

JOHNSON, J.D., EPEL, D., & PAUL, M. (1976) Intracellular pH and activation of sea urchin eggs after fertilization. *Nature (Lond.),* **262**(5570): 661-664.

JONES, K. (1973) Ammonia. In: Bailar, J.C., Jr, Emeleus, H.J., Nyholm, R., & Trotman-Dickenson, A.F., ed. *Comprehensive inorganic chemistry,* New York, Pergammon Press, Vol. 2, pp. 199-227.

JONES, R.D. & HOOD, M.A. (1980) Effects of temperature, pH, salinity and inorganic nitrogen on the rate of ammonium oxidation by nitrifiers isolated from wetland environments. *Microb. Ecol.,* **6**: 339-347.

JUDE, D.J. (1973) *Sublethal effects of ammonia and cadmium on growth of green sunfish,* East Lansing, Michigan, Michigan State University, 193 pp (Ph.D. Thesis).

KADOWAKI, S. (1976) Size distribution of atmospheric total aerosols, sulfate, ammonium and nitrate particulates in the Nagoya area. *Atmos. Environ.,* **10**: 39-43.

KAGOTA, K., IWASE, T., KOJIMA, T., NIIYAMA, M., & NAMIOKA, S. (1979) Diammonium citrate addition to a diet restricted non-essential amino acids for young pigs. *Jap. J. vet. Sci.,* **41**: 131-138.

KAMIN, H. & HANDLER, P. (1957) Amino acid and protein metabolism. *Annu. Rev. Biochem.,* **26**: 419-490.

KAPEGHIAN, J.C., MINCER, H.H., JONES, A.B., VERLANGIERI, A.J., & WATERS, I.W. (1982) Acute inhalation toxicity of ammonia in mice. *Bull. environ. Contam. Toxicol.,* **29**: 371-378.

KARNAKY, K.J., Jr, KINTER, L.B., KINTER, W.B., & STIRLING, C.E. (1976) Teleost chloride cell. II. Autoradiographic localization of gill Na, K-ATPase in killifish *Fundulus heterclitus* adapted to low and high salinity environments. *J. cell Biol.,* **70**: 157-177.

KATZ, M. & PIERRO, R.A. (1967) *Estimates of the acute toxicity of ammonia-urea plant wastes to coho salmon,* Oncorhynchus kisutch, Seattle, Washington, Fisheries Research Institute, College of Fisheries, University of Washington, 15 pp (Final report).

KEESEE, R.G., HOPF, S.B., & MOYERS, J.L. (1975) The distribution and characteristics of particulate sulfates in the southwest desert atmosphere. Presented at: The *International Conference on Environmental Sensing and Assessment, Las Vegas, Nevada, 14-19 September, 1975,* Vol. 2, 5 pp (Paper 23-6).

KEMPINSKA, H. (1968) [The effect of ammonia fertilizers on fish.] *Gospod. Rybn.,* **20**: 3-5 (in Russian).

KEPLINGER, M.L., SCHADEBERG, K.J., GOODE, J.W., & CALANDRA, J.C. (1973) Irritation threshold evaluation study with ammonia. In: *Report to the Institute of Ammonia Refrigeration,* Northbrook, Illinois, Industrial Bio-Test Laboratories, Inc.

KERN, D.M. (1960) The hydration of carbon dioxide. *J. Chem. Ed.,* **37**: 14-23.

KERSTETTER, T.H., KIRSCHNER, L.B., & RAFUSE, D.D. (1970) On the mechanisms of sodium ion transport by the irrigated gills of rainbow. *J. gen. Physiol.,* **56**: 342-359.

KEYES, W.F. (1975) Nitrogen. In: *Mineral facts and problems,* Washington DC, US Department of the Interior, pp. 749-760 (Bureau of Mines Bulletin No. 667).

KHOLDEBARIN, B. & OERTLI, J.J. (1977) Effect of pH and ammonia on the rate of nitrification of surface water. *J. Water Pollut. Control Fed.,* **49**: 1693-1697.

KIRKPATRICK, W.C., ROLLER, M.H., & SWANSON, R.N. (1972) Serum- and tissue-ammonia nitrogen and tissue-water values in

ammonia-intoxicated sheep. *Am. J. Vet. Res.,* **33**: 1187-1190.

KIRKPATRICK, W.C., ROLLER, M.H., & SWANSON, R.M. (1973) Hemogram of sheep acutely intoxicated with ammonia. *Am. J. Vet. Res.,* **34**: 587-589.

KIRSCHNER, L.B., GREENWALD, L., & KERSTETTER, T.H. (1973) Effect of amiloride on sodium transport across body surfaces of freshwater animals. *Am. J. Physiol.,* **224**: 832-837.

KLEMM, K. (1967) The effect of N source on the yield of various crop plants. *Bodenkultur,* **18**: 210-218.

KLENDSHOJ, N.C. & REJENT, T.A. (1966) Tissue levels of some poisoning agents less frequently encountered. *J. Forensic Sci.,* **11**: 75-80.

KLING, H.F. & QUARLES, C.L. (1974) Effect of atmospheric ammonia and the stress of infectious bronchitis vaccination on Leghorn males. *Poult. Sci.,* **53**: 1161-1167.

KLUESENER, J.W. & LEE, G.F. (1974) Nutrient loading from a separate storm sewer in Madison, Wisconsin. *J. Water Pollut. Control Fed.,* **46**(5): 920-936.

KNEPP, G.L. & ARKIN, G.F. (1973) Ammonia toxicity levels and nitrate tolerance of channel catfish. *Prog. Fish-Cult.,* **35**(4): 221-224.

KNOWLES, G., DOWNING, A.L., & BARRETT, M.J. (1965) Determination of kinetic constants for nitrifying bacteria in mixed culture, with the aid of an electronic computer. *J. gen. Microbiol.,* **38**: 263.

KOENIG, H. & KOENIG, R. (1949) Production of acute pulmonary edema by ammonium salts. *Proc. Soc. Exp. Biol. Med.,* **70**: 375-380.

KOPP, A., FELLER, U., & ERISMANN, K.H. (1974) [Studies on the regulation of nitrogen assimilation by *Lemna minor* in transition from ammonium to nitrate feeding or from nitrate to ammonium feeding under conditions of photosynthesis.] *Z. Pflanzen physiol.,* **73**: 456-460 (in German).

KORMANIK, G.A. & CAMERON, J.N. (1981) Ammonia excretion in the FW catfish: the role of diffusion. *Am. Soc. Zool.,* **21**(4): 1042.

KORTING, W. (1969a) [The harmful effect of ammonia on fish.] *Munch. Beitr. Abwasser-. Fisch.-Flussbiol.,* **16**: 38-48 (in German).

KORTING, W. (1969b) [Investigations on the effects of ammonia on the blood of fish.] *Wasser Abwasser Forsch.,* **4**: 154-159 (in German).

KRYSTAL, G.W. & POCCIA, D. (1979) Control of chromosome condensation in the sea urchin egg. *Exp. cell Res.,* **123**(2): 207-219.

KULASEK, G., SIWECKA, B., OWCZARCZYK, B., RYNCA, J., & BAREJ, W. (1975) Effect of protein level and synthesize chloride addition to diet on ammonia detoxification in rats. *Zentralbl. Veterinaermed.,* **22**(3): 215-223.

KUSHNIR, J.J., MALKIN, H.I., MOHNEN, V.A., JENCHA, A.J., & MCLAREN, E.H. (1970) *Non-photochemical aerosol formation from the anhydrous reaction between ammonia and sulfur dioxide gases. Mechanism for the formation of ammonium sulfate in the atmosphere,* Albany, New York, Department of Chemistry and Atmospheric Sciences Research Center, State University of New York (Report 165 of National Air Pollution Control Administration Research Grant 5RO1A).

KUSTOV, V.V. (1967) [Means of determining the maximum allowable concentration of toxic products of natural human metabolism.] In: *General questions of industrial toxicology,* Moscow, pp. 63-65. (National Aeronautics and Space Administration Technical Translation NASA TT F-11,358) (in Russian).

LANDAHL, H.D. & HERRMANN, R.G. (1950) Retention of vapors and gases in the human nose and lung. *Arch. ind. Hyg. occup. Med.,* **1**: 36-45.

LANGOWSKA, I. & MOSKAL, J. (1974) Effect of ammonia and urea on nitrobacteria in water environment. *Pol. Arch. Hydrobiol.,* **21**(1): 119-123.

LARSON, T.V., COVERT, D.S., FRANK, R., & CHARLSON, R.J. (1977) Ammonia in the human airways: neutralization of inspired acid sulfate aerosols. *Science,* **197**: 161-163.

LECHTENBERG, V.L., BUETTNER, M.R., HOLT, D.A., RICHEY, C.B., & PARSONS, S.D. (1977) Hay preservation by anhydrous ammonia treatment. In: *Grain and Forage Harvesting. Proceedings of the First International Grain and Forage Conference,* St. Joseph, American Society of Agricultural Engineers, pp. 327-328, 338.

LEE, J.A., (1985) Effects of NO_2 on aquatic ecosystems. In: *Study on the need of NO_2 long term limit value for the protection of terrestrial and aquatic ecosystems* (Final Report Feb. 1985).

LEE, R.E., Jr & GORANSON, S. (1976) National air surveillance cascade impact network. III. Variations in size of airborne particulate matter over a three-year period. *Environ. Sci. Technol.,* **10**: 1022-1027.

LEITHE, W. (1971) *The analysis of air pollutants,* Ann Arbor, Michigan, Ann Arbor Science Publishers.

LEONARDOS, G., KENDALL, D., & BARNARD, N. (1969) Odour threshold determination of 52 odorant chemicals. *J. Air Pollut. Control Assoc.,* **19**: 91-95.

LEUVEN, R.S.E.W. & SCHUURKES, J.A.A.R. (1984) *[Effects of acid precipitation containing sulfur and nitrogen on weakly buffered waters low in nutrients]* The Netherlands, Laboratory of Aquatic Ecology, University of Nymegen, and Ministry of Housing, Physical Planning and Environment, 131 pp (in Dutch).

LEVI, G., MORISI, G., COLETTI, A., & CATANZARO, R. (1974) Free amino acids in fish brain: normal levels and changes upon exposure to high ammonia concentrations *in vivo,* and upon incubation of brain slices. *Comp. Biochem. Physiol.,* **49A**: 623-636.

LEVY, D.M., DIVERTIE, M.B., LITZOW, T.J., & HENDERSON, J.W. (1964) Ammonia burns of the face and respiratory tract. *J. Am. Med. Assoc.,* **190**: 873-876.

LIEBHARDT, W.C., GOLT, C., & TUPIN, J. (1979) Nitrate and ammonium concentrations of ground water resulting from poultry manure applications. *J. environ. Qual.,* **8**: 211-215.

LIECHTI, P.M. & HUGGINS, D.G. (1980) Effects of a 24-h ammonia injection on stream drift and benthic standing crop. *Trans. Kansas Acad. Sci.,* **83**(3): 140.

LIETZKE, M.H. (1978) A kinetic model for predicting the composition of chlorinated water discharged from power plant cooling systems. In: *Water chlorination environmental impact and health effects,* Ann Arbor, Michigan, Ann Arbor Science Publishers, Inc., Vol. 2, pp. 707-716.

LILLIE, R.J. (1970) Ammonia. In: *Air pollutants affecting the performance of domestic animals. A literature review,* Washington DC (Agriculture Handbook No 380).

LINDEN, E., BENGTSSON, B.-E., SVANBERG, O., & SUNDSTROM, G. (1979) The acute toxicity of 78 chemicals and pesticide formulations against two brackish water organisms, the bleak *(Alburnus alburnus)* and the harpacticoid *(Nitocra spinipes). Chemosphere,* **11**(12): 843-851.

LINZON, S.N. (1971) Effects of air pollutants on vegetation. In: McCormac, B.M., ed. *Introduction to the scientific study of atmospheric pollution,* Dordrecht, Holland, D. Reidel Publishing Company, pp. 131-151.

LITAV, M. & AGAMI, M. (1976) Relationship between water pollution and the flora of two coastal rivers of Israel. *Aquat. Biol.,* **2**(1): 23-41.

LITAV, M. & LEHRER, Y. (1978) The effects of ammonium in water on *Potamageton lucens. Aquat. Biol.,* **5**(2): 127-138.

LITTON BIONETICS, INC. (1975) *Mutagenic evaluation of compound FDA 73-42 ammonium sulfate, granular, food grade,* Washington DC, Litton Bionetics (Prepared for US Food and Drug Administration) (NTIS PB-245 506).

LLOYD, R. (1961) Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout (*Salmo gairdnerii* Richardson). *J. exp. Biol.,* **38**: 447-455.

LLOYD, R. & HERBERT, D.W.M. (1960) The influence of carbon dioxide on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdnerii* Richardson). *Ann. appl. Biol.,* **48**(2): 399-404.

LLOYD, R. & ORR, L.D. (1969) The diuretic response by rainbow trout to sub-lethal concentrations of ammonia. *Water Res.,* **3**(5): 335-344.

LOBASHOV, M.E. (1937) [Nature of the effects of chemical agents on the mutation process of *Drosphila melanogaster.] Genetica, **19**: 200-241 (in German).*

LOBASHOV, M.E. & SMIRNOV, F.A. (1934) [Effect of acetic acid on nondisjunction and mutations in *Drosphila melanogaster.*]

Dokl. Acad. Sci. USSR N.S., **2**: 307 (in Russian with German summary).

LODGE, J.P., Jr, MACHADO, P.A., PATE, J.B., SHEESLEY, D.C., & WARTBURG, A.F. (1974) Atmospheric trace chemistry in the American humid tropics. *Tellus,* **26**: 250-253.

LOEHR, R.C. (1974) Characteristics and comparative magnitude of non-point sources. *J. Water Pollut. Control Fed.,* **46**(8): 1849-1872.

LOSADA, M. & ARNON, D.I. (1963) Selective inhibitors of photosynthesis. In: Hochester, R. & Quastel, J.H., ed. *Metabolic inhibitors,* New York, Academic Press, Vol. 2, p. 559.

LOSADA, M., HERRERA, J., MALDONADO, J.M., & PANEQUE, A. (1973) Mechanism of nitrate reductase reversible inactivation by ammonia in *Chlamydomonas. Plant Sci. Lett.,* **1**: 31-37.

LOTSPEICH, W.D. (1965) Renal hypertrophy in metabolic acidosis and its relation to ammonia excretion. *Am. J. Physiol.,* **208**(6): 1135-1142.

LUBINSKI, K.S. (1979) *Monitoring bluegill swimming behaviour and the effects of sublethal ammonium chloride gradients,* Blacksburg, Virginia, Virginia Polytechnic Institute and State University, 99 pp (Ph.D. Thesis).

LUBINSKI, K.S., SPARKS, R.E., & JAHN, L.A. (1974) *The development of toxicity indices for assessing the quality of the Illinois River,* Urbana, Illinois, Water Resources Center, University of Illinois, 46 pp (Research Report No. 96).

LUBINSKI, K.S., DICKSON, K.L., & CAIRNS, J., Jr (1980) Effects of abrupt sublethal gradients of ammonium chloride on the activity level, turning, and preference-avoidance behaviour of bluegills, In: Eaton, J.G., Parrish, P.R., & Hendricks, A.C. ed. *Aquatic toxicology,* Philadelphia, Pennsylvania, American Society for Testing and Materials, pp. 328-340 (ASTM STP 707).

MACEWEN, J.D. & VERNOT, E.H. (1972) *Toxic hazards research unit annual technical report* (NTIS AD 755-358).

MACEWEN, J.D., THEODORE, J., & VERNOT, E.H. (1970) Human exposure to EEL concentrations of monomethylhydrazine. In: *Proceedings of the 1st Annual Conference on Environmental Toxicology, Ohio, Wright-Patterson Air Force Base, 9-11 September, 1970,* Aerospace Medical Research Laboratory, pp. 355-363 (AMRL-TR-70-102, Paper No. 23).

MCBARRON, E.J. & MCINNES, P. (1968) Observations on urea toxicity in sheep. *Aust. Vet. J.,* **44**: 90-96.

MCBEAN, R.L., NEPPEL, M.J., & GOLDSTEIN, L. (1966) Glutaminate dehydrogenase and ammonia production in the eel *(Anguilla rostrata). Comp. Biochem. Physiol.,* **18**: 909-920.

MCBRIDE, R.L. & LAING, D.G. (1979) Threshold determination by triangle testing: Effects of judgmental procedure, positional bias and incidental training. *Chem. Senses Flavor.,* **4**(4): 319-328.

MCCALLAN, S.E.A. & SETTERSTROM, C. (1940) Toxicity of

ammonia, chlorine, hydrogen cyanide, hydrogen sulphide, and sulphur dioxide gases. I. General methods and correlations. *Contrib. Boyce Thompson Inst.,* **11**: 325-330.

MCCAY, C.M. & VARS, H.M. (1931) Studies upon fish blood and its relation to water pollution. In: *A biological survey of the St. Lawrence Watershed. Supplement to the 20th Annual Report, 1930,* Albany, New York, New York Conservation Department, pp. 230-233 (Biol. Survey (1930), No. V).

MCCORMICK, J.H., BRODERIUS, S.J., & FIANDT, J.T. (1984) Toxicity of ammonia to early life stages of the green sunfish *Lepomis cyanellus. Environ. Pollut. Ser. A.*, **36**: 147-163. *Erratum: Environ. Pollut. Ser. A.* (in press).

MCDONALD, D.G. & WOOD, C.M. (1981) Branchial and renal acid and ion fluxes in the rainbow trout at low environmental pH. *J. exp. Biol.,* **93**: 101-118.

MCKAY, H.A.C. (1969) Ammonia and air pollution. *Chem. Ind.,* **34**: 1162-1165.

MCKHANN, G.M. & TOWER, D.B. (1961) Ammonia toxicity in cerebral oxidative metabolism. *Am. J. Physiol.,* **200**: 420-424.

MAETZ, J. (1972) Branchial sodium exchange and ammonia excretion in the goldfish *Carassius auratus.* Effects of ammonia-loading and temperature changes. *J. exp. Biol.,* **56**: 601-620.

MAETZ, J. (1973) Na^+/NH_4+, Na^+/H^+ exchanges and NH_3 movement across the gill of *Carassius auratus. J. exp. Biol.,* **58**: 255-275.

MAETZ, J. & GARCIA-ROMEU, F. (1964) The mechanism of sodium and chloride uptake by the gills of a freshwater fish, *Carassius auratus.* II Evidence for NH_4^+/Na^+ and HCO_3-/Cl^- exchanges. *J. gen. Physiol.,* **47**: 1209-1227.

MAGALHAES BASTOS, J.A. (1954) *[Importance of ammonia as an ichthyotoxic substance,]* Brazil, Serv. Piscicultura, Dept. nacl. obras contra secas, Ministerio viacao e obras publicas, pp. 115-132 (Publ. Ser. 1-C, No. 159) (in Portuguese).

MAI, K.L. (1977) Energy and petrochemical raw materials through 1990. *Chem. Eng.,* **84**: 122-128.

MALACEA, I. (1966) [Contributions to knowledge of the cyanide, ammonia, mercury and arsenic toxic action on some species of fishes and on *Daphnia.] Stud. Prot. Epurarea Apelor Inst. Stud. Cercet Hidrotehnice,* **7**(2: 751-792 (English summary) *Biol. Abstr.,* **50**: 92715 (1969).

MALACEA, I. (1968) [Studies on the acclimatization of fish to high concentrations of toxic substances.] *Arch. Hydrobiol.,* **65**(1): 74-95 (in German with English summary).

MALLERY, C.H. (1979) Ammonium stimulated properties of a K^+-dependent ATPase in *Opsanus beta,* a marine teleost with an NH_4+/Na^+ exchange pump. *Am. Zool.,* **19**: 944.

MANGOLD, C.A. (1971) *Investigation of occupational exposure to ammonia,* Washington, Puget Sound Naval Shipyard, Industrial Hygiene Division of Investigation.

MANGUM, C.P., SILVERTHORN, S.U., HARRIS, J.L., TOWLE, D.W., & KRALL, A.R. (1976) The relationship between blood pH, ammonia excretion and adaptation to low salinity in the blue crab *Callinectes sapidus. J. exp. Zool.,* **195**(1): 129-136.

MANGUM, C.P., DYKENS, J.A., HENRY, R.P., & POLITES, G. (1978) The excretion of NH_4+ and its ouabain sensitivity in aquatic annelids and molluscs. *J. exp. Zool.,* **203**: 151-157.

MARSCHANG, F. (1972) [The NH_3 content of air in stables and the fattening capacity of cows.] *Dtsch. Tieraerztl. Wochenschr.,* **79**: 214-216 (in German).

MARSCHANG, F. & CRAINICEANU, E. (1971) [Investigations on the connections between cowshed climate and calf mortality.] *Berlin München Tieraerztl. Wochenschr.,* **84**: 417-420 (in German).

MARSCHANG, F. & PETRE, C. (1971) [The NH_3 content of cowshed air and its influence on morbidity and animal lossess in cattle-fattening pens.] *Zentralbl. Veterinaermed.,* **18**: 646-654 (in German).

MATASA, C. & MATASA, E. (1968) *L'industrie moderne des produits azotés,* Paris, Dunod, pp. 63-208.

MATSUMOTO, H., OKAMURA, K., & TAKAHASHI, E. (1976) Changes in activity and isoenzymes of intervase in cucumber leaves due to nitrogen status. *Plant Cell Physiol.,* **17**: 867-874.

MATUSIAK, K. (1976) Studies on the purification of wastewater from the nitrogen fertilizer industry by intensive algal cultures. *Acta microbiol. Pol.,* **25**(3): 233-242.

MAYAN, M.H. & MERILAN, C.P. (1972) Effects of ammonia inhalation on respiratory rate of rabbits. *J. anim. Sci.,* **34**: 448-452.

MAYNARD, D.N. & BARKER, A.V. (1969) Studies on the tolerance of the plants to ammonium nutrition. *J. Am. Soc. Hortic. Sci.,* **94**: 235-239.

MEARNS, A.J. (1981) Ecological effects on ocean sewage outfalls: observations and lessons. *Oceanus,* **24**: 45-54.

MERKENS, J.C. & DOWNING, K.M. (1957) The effect of tension of dissolved oxygen on the toxicity of un-ionized ammonia to several species of fish. *Ann. appl. Biol.,* **45**(3): 521-527.

METCALF & EDDY, INC. (1972) *Wastewater engineering,* New York, McGraw-Hill.

MILES, F.D. (1961) *Nitric acid manufacture and uses,* London, Oxford University Press.

MILLER, T.J. (1973) Cleft palate formation: The effects of fasting and iodoacetic acid on mice. *Teratology,* **7**: 177-182.

MILLER, G.R., SHIMP, G.F., ANDREWS, H.O., NIMMO, D.W., & ILEY, E.S. (1981) Effect of domestic wastewater on trout in Dillon Reservoir, Colorado. Paper presented at: *The ASTM Symposium on Aquatic Toxicology, St. Louis, Missouri, 13-14 October 1981,* Philadelphia, Pennsylvania, American Society for Testing

Materials.

MINER, J.R. & HAZEN, T.E. (1969) Ammonia and amines: components of swine-building odour. *Trans. Am. Soc. Agric. Eng.,* **12**: 772-774.

MITCHELL, H.A. (1963) Ammonia tolerance of the California leaf-nosed bat. *J. Mammal,* **44**: 543-551.

MITCHELL, H.A. (1964) Investigations of the cave atmosphere of a Mexican bat colony. *J. Mammal,* **45**: 568-577.

MORII, H., NISHIKATA, K., & TAMURA, O. (1978) Nitrogen excretion of mudskipper fish *Periophthalmus cantonensis* and *Boleophthalmus pectinirostris* in water and on land. *Comp. Biochem. Physiol.,* **60A**(2): 189-193.

MORRIS, J.C. (1967) Kinetics of reactions between aqueous chlorine and nitrogenous compounds. In: Faust, S.D. & Hunter, J.V., ed. *Principles and applications of water chemistry,* New York, John Wiley and Sons, pp. 23-53.

MORRIS, J.C. (1978) Chemistry of aqueous chlorine in relation to water chlorination. In: Jolley, R.L., ed. *Water chlorination: Environmental impact and health effects,* Ann Arbor, Michigan, Ann Arbor Science Publishers, Vol. 1, pp. 21-36.

MORRIS, J.C. & ROGERS, Q.R. (1978) Ammonia intoxication in near-adult cat as a result of a dietary deficiency of arginine. *Science,* **199**(4327): 431-432.

MOSIER, A.R. (1978) Inhibition of photosynthesis and nitrogen fixation in algae by volatile nitrogen bases. *J. environ. Qual.,* **7**(2): 237-240.

MOSSBERG, S.M. (1967) Ammonia absorption in hamster iluem. Effect of pH and total CO_2 on transport in everted sacs. *Am. J. Physiol.,* **213**: 1327-1330.

MOSSBERG, S.M. & ROSS, G. (1967) Ammonia movement in the small intestine: Preferential transport by the ileum. *J. clin. Invest.,* **46**: 490-498.

MOTYL, T. & DEBSKI, B. (1977) The influence of increasing amounts of NH_4Cl in the diet on ammonia detoxification in rats. *Zbl. Vet. Med.,* **24**: 333-342.

MULDER, J.S. & VAN DER ZALM, H.O. (1967) [A fatal case of ammonia poisoning.] *Tijdschr. Soc. Geneeskd.,* **45**: 458-460 (in Dutch).

MULLER, G. & GRUHN, M. (1969) [The effect of water-free ammonia fertilizer on soil microorganisms.] *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 2 Naturw.,* **123**: 667-676 (in German with English summary).

MUNTWYLER, E., IACOBELLIS, M., & GRIFFIN, G.E. (1956) Kidney glutaminase and carbonic anhydrase activities and renal electrolyte excretion in rats. *Am. J. Physiol.,* **184**: 83-90.

NAKAZAWA, S. & QUASTEL, J.H. (1968) Inhibitory effects on ammonium ions and some amino acids on stimulated brain respiration and cerebral amino acid transport. *Can. J.*

Biochem., **46**: 543-548.

NATARAJAN, K.V. (1970) Toxicity of ammonia to marine diatoms. *J. Water Pollut. Control Fed.,* **42**(5, part 2): R184-R190.

NAVAZIO, F., GERRITSEN, T., & WRIGHT, G.J. (1961) Relationship of ammonia intoxication to convulsions and coma in rats. *J. Neurochem.,* **8**: 146-151.

NEHRING, D. (1963) [The toxic effect of urea solutions containing urease on various fish species.] *Z. Fischerei,* **11**(7/8): 539-547 (in German).

NEUFELD, R.D., HILL, A.J., & ADEKOYA, D.O. (1980) Phenol and free ammonia inhibition to Nitrosomonas activity. *Water Res.,* **14**(12): 1695-1703.

NIDEN, A.H. (1968) Effects of ammonia inhalation of the terminal airways. In: *Proceedings of the 11th Aspen Emphysema Conference,* Vol. 11, pp. 41-44.

NIHLGARD, B. (1970) Precipitation, its chemical composition and effects on soil water and beech and spruce forests in South-Sweden. *Oikos.,* **21**: 208

NIHLGARD, B. (1985) The ammonium hypothesis: an additional explanation to the forest dieback in Europe. *Ambio,* **14**(1): 1-8.

NISHIOKA, D.J. (1976) *The initiation of cell cycle events in ammonia-treated eggs and merogons of the sea urchin,* Berkeley, California, University of California, 107 pp (Ph.D. Thesis).

NODA, K. & CHIKAMORI, K. (1976) Effect of ammonia via prepyriform cortex on regulation of food intake in the rat. *Am. J. Physiol.,* **231**: 1263-1266.

NRC (1979) *Ammonia. Subcommittee on Ammonia. Committee on Medical and Biologic Effects of Environmental Pollutants. Division of Medical Sciences, Assembly of Life Sciences, National Research Council,* Baltimore, Maryland, University Park Press (NTIS No. PB 278-027).

OKITA, T. & KANAMORI, S. (1971) Determination of trace concentration of ammonia in the atmosphere using pyridine-pyrazolone reagent. *Atmos. Environ.,* **5**: 621-627.

OVERREIN, L.N., SEIP, H.M., & TOLLAN, A., ed. (1980) *Acid precipitation: effects on forest and fish,* Oslo (Final report SNSF Project 1972-80).

PAIK, W.K., LAWSON, D., & KIM, S. (1975) Effect of endocrine glands on the protection of rat from ammonia intoxication. *Life Sci.,* **15**: 1189-1196.

PARKHURST, B.R., BRADSHAW, A.S., FORTE, J.L., & WRIGHT, G.P. (1979) An evaluation of the acute toxicity to aquatic biota of a coal conversion effluent and its major components. *Bull. environ. Contam. Toxicol.,* **23**: 349-356.

PARKHURST, B.R., MEYER, J.S., DEGRAEVE, G.M., & BERGMAN, H.L. (1981) A re-evaluation of the toxicity of coal conversion process waters. *Bull. environ. Contam. Toxicol.,* **26**: 9-15.

PAUL, M., JOHNSON, J.D., & EPEL, D. (1976) Fertilization acid of sea urchin eggs is not a consequence of cortical granule exocytosis. *J. exp. Zool.,* **197**(1): 127-133.

PAUL, M. & JOHNSTON, R.N. (1978) Absence of a Ca response following ammonia activation of sea urchin eggs. *Dev. Biol.,* **67**(2): 330-335.

PAYAN, P. (1978) A study of the Na^+/NH_4^+ exchange across the gill of the perfused head of the trout *(Salmo gairdneri). J. comp. Physiol.,* **124**: 181-188.

PAYAN, P. & MAETZ, J. (1973) Branchial sodium transport mechanisms in *Scyliorhinis canicula:* evidence for Na^+/NH_4^+ and Na^+/H^+ exchanges and for a role of carbonic anhydrase. *J. exp. Biol.,* **58**: 487-502.

PAYAN, P. & MATTY, A.J. (1975) The characteristics of ammonia excretion by a perfused isolated head of trout *(Salmo gairdneri)*: effect of temperature and CO_2-free ringer. *J. comp. Physiol.,* **96**: 167-184.

PAYAN, P., MATTY, A.J., & MAETZ, J. (1975) A study of the sodium pump in the perfused head preparation of the trout, *Salmo gairdneri* in freshwater. *J. comp. Physiol.,* **104**: 33-48.

PEPLINSKI, A.J., BREKKE, O.L., BOTHAST, R.J., & BLACK, L.T. (1978) High moisture corn: an extended preservation trial with ammonia. *Trans. Am. Soc. Agricul. Eng.,* **21**: 773-776, 781.

PEQUIN, L. & SERFATY, A. (1963) L'excrétion ammoniacale chez un téléostéen dulcicole *Cyprinus carpio. J. comp. Biochem. Physiol.,* **10**: 315-324.

PERRY, J.H., CHILTON, C.H., & KIRKPATRICK, S.D., ed. (1963) *Chemical engineers' handbook*, 4th ed., New York, McGraw-Hill, pp. 3-151.

PHILLIPS, A.M. (1950) *The effect of metabolic products on brook trout,* Cortland, New York, New York State Conservation Department, pp. 14-16 (Fisheries Research Bulletin 14) (Cortland Hatcherry Report 19).

PHILLIPS, R.W. (1970) Further clinical studies of trimethylpuringyl ammonium chloride (NSA-51095) in malignant disease with special emphasis on cancer of urinary bladder. *Cancer Chemother. Rep.,* **54**: 181.

PIERPONT, R.A. & MINOTTI, P.L. (1977) Effects of calcium carbonate on ammonium assimilation by tomato seedlings. *J. Am. Soc. Hortic. Sci.,* **102**: 20-23.

PINTER, I.J. & PROVASOLI, L. (1963) Nutritional characteristics of some chrysomonads. In: Oppenheimer, C.M., ed. *Symposium on Marine Microbiology,* Springfield, Massachusetts, Thomas Publishers, pp. 114-121.

PITTS, R.F. (1971) The role of ammonia production and excretion in regulation of acid-base balance. *New Engl. J. Med.,* **284**: 32-38.

PITTS, J.N., Jr & GROSJEAN, D. (1976) *Detailed characterization of gaseous and size-resolved particulate pollutants at a south coast air basin smog receptor site:*

levels and modes of formation of sulfate, nitrate and organic particulates and their implications for control strategies, Riverside, California, Statewide Air Pollution Research Center, University of California (California Air Resources Board Contract 5-384, Six Month Progress Report, November 17, 1975 to May 16, 1976).

POLIAK, V.E. (1981) [Air pollution by ammonia around swine-breeding complexes.] *Gig. i. Sanit.,* **6**: 67 (in Russian).

POST, R.L. & JOLLY, P.C. (1957) The linkage of sodium, potassium and ammonium active transport across the human erythrocyte membrane. *Biochem. Biophys. Acta,* **25**: 118-128.

POWERS, E.B. (1920) Influence of temperature and concentration on the toxicity of salts to fishes. *Ecology,* **1**: 95-112.

POWERS, W.L., TERRY, R.V., & MURPHY, L.S. (1977) Fate of nitrogen from manure disposal. In: *Proceedings of the National Conference on Disposal of Residues on Land,* pp. 156-160.

PROPKOPIEVA, A.S. & YUSHKOV, G.G. (1975) [Toxicology of ammonia in animals repeatedly exposed for short lengths of time.] *Gig. Tr. Prof. Zabol.,* **9**: 54-55 (in Russian).

PROPKOPIEVA, A.S., YUSHKOV, G.G., & UBASHEEV, I.O. (1973) [Materials for a toxicological characteristic of the one-term effect of ammonia on the organisms of animals after brief exposure.] *Gig. Tr. Prof. Zabol.,* **6**: 56-57 (in Russian).

PROVASOLI, L. & MCLAUGHLIN, J.J.A. (1963) Limited heterotrophy of some photosynthetic dinoflagellates. In: Oppenheimer, C.M., ed. *Symposium on Marine Microbiology,* Springfield, MA, Thomas Publishers, pp. 105-113.

PRZYTOCKA-JUSIAK, M. (1976) Growth and survival of *Chlorella vulgaris* in high concentrations of nitrogen. *Acta microbiol. Pol.,* **25**(3): 287-289.

PRZYTOCKA-JUSIAK, M., MLYNARCZYK, A., KULESZA, M., & MYCIELSKI, R. (1977) Properties of *Chlorella vulgaris* strain adapted to high concentrations of ammonium nitrogen. *Acta microbiol. Pol.,* **26**(2): 185-197.

QUARLES, C.L. & KLING, H.F. (1974) Evaluation of ammonia and infectious bronchitis vaccination stress on broiler performance and carcass quality. *Poult. Sci.,* **53**: 1592-1596.

RAJAGOPAL, R. (1978) Impact of land use on ground water quality in the Grand Traverse Bay region of Michigan. *J. environ. Qual.,* **7**: 93-98.

RAMACHANDRAN, V. & RAMAPRABHU, T. (1976) Use of ammonia as herbicide: A Review. In: Varshney, C.K. & Rzoska, J., ed. Aquatic weeds in South East Asia, The Hague, B.V. Publishers, pp. 293-298.

RAMACHANDRAN, V., RAMAPRABHU, T., & REDDY, P.V.G.K. (1975) Observations on the use of ammonia for the control of Pistia stratiotes Linn. *J. Int. Fish. Soc. India*, **7**: 124-130.

RAMSEY, G.B. (1953) Mechanical and chemical injuries. In: *The yearbook of agriculture 1953,* Washington DC, US Department of Agriculture, Plant Diseases, pp. 835-837.

RANDALL, D.J. (1970) Gas exchange in fish. In: Hoar, W.S. & Randall, D.J., ed. *Fish physiology,* New York, Academic Press, Vol. IV., pp. 253-292.

RANKIN, D.P. (1979) The influence of un-ionized ammonia on the long-term survival of sockeye salmon eggs. *Fish. Mar. Serv. Tech. Rep.,* **912**: 17.

RAO, T.S., RAO, M.S., & PRASAD, S.B.S.K. (1975) Median tolerance limits of some chemicals to the fresh water fish *"Cyprinus carpio". Indian J. environ. Health,* **17**(2): 140-146.

REDNER, B.D. & STICKNEY, R.R. (1979) Acclimatization to ammonia by *Tilapia aurea. Trans. Am. Fish Soc.,* **108**(4): 383-388.

REICHENBACH-KLINKE, H.-H. (1967) [Investigations on the influence of the ammonia content on the fish organism.] *Arch. Fischereiwiss.,* **17**(2): 122-132. (in German).

REINBOLD, K.A. & PESCITELLI, S.M. (1982a) *Effects of exposure to ammonia on sensitive life stages of aquatic organisms,* Champaign, Illinois, Illinois Natural History Survey (Project Report: Contract No. 68-01-5832).

REINBOLD, K.A. & PESCITELLI, S.M. (1982b) *Effects of cold temperature on toxicity of ammonia to rainbow trout, blue gills, and fathead minnows,* Champaign, Illinois, Illinois Natural History Survey (Project Report: Contract No. 68-01-5832).

REINBOLD, K.A. & PESCITELLI, S.M. (1982c) *Acute toxicity of ammonia to the white sucker,* Champaign, Illinois, Illinois Natural History Survey, 11 pp (EPA Contract No. 2W-3946 NAEX).

REINBOLD, K.A. & PESCITELLI, S.M. (1982d) *Acute toxicity of ammonia to channel catfish,* Champaign, Illinois, Illinois Natural History Survey, 11 pp (EPA Contract No. J 2482 NAEX).

REITER, R., SLADKOVIC, R., & POTZL, K. (1976) Chemical components of aerosol particles in the lower troposphere above central Europe measured under pure-air conditions. *Atmos. Environ.,* **10**: 841-853.

REMPEL, B. (1975) Use ammonia to stop corn deterioration. *Crops Soils,* **27**: 13-14.

REPP, W.W., HALE, W.H., CHENG, E.W., & BURROUGHS, W. (1955) The influence of oral administration of non-protein nitrogen feeding compounds upon blood ammonia and urea levels in lambs. *J. anim. Sci.,* **14**: 118-131.

RICE, S.D. & BAILEY, J.E. (1980) Survival, size, and emergence of pink salmon, *Oncorhynchus gorbuscha,* alevins after short- and long-term exposures to ammonia. *Fish Bull.,* **78**(3): 641-648.

RICHARD, D., BOULEY, G., & BOUDENE, C. (1978a) Effets de l'inhalation continue d'ammoniac chez le rat et la souris. *Bull. eur. Physiopathol. resp.,* **14**: 573-582.

RICHARD, D., JOUARY, J., & BOUDENE, C. (1978b) Toxicité aiguë par voie aérienne du gaz ammoniac chez le lapin.

C.R. Acad. X. Paris, **287**(Série D): 375-378.

RICHARDS, B.O. & FROMM, P.O. (1970) Sodium uptake by isolated perfused gills of rainbow trout *(Salmo gairdneri). Comp. Biochem. Physiol.,* **33**: 303-310.

RICHARDS, P., HOUGHTON, B.J., METCALFE-GIBSON, A., WARD, E.E., & WRONG, O. (1968) Incorporation of orally administered ammonia into tissue proteins in man; the influence of diet and ureamia. In: *Nutrition and renal disease,* Edinburgh, E. and S. Livingstone, pp. 93-98.

RICHARDS, P., BROWN, C.L., HOUGHTON, B.J., & WRONG, D.M. (1975) The incorporation of ammonia nitrogen into albumin in man: The effects of diet, uremia and growth hormone. *Clin. Nephrol.,* **3**(5): 172-179.

RINGER, R.K. (1971) Adaptation of poultry to confinement rearing systems. *J. anim. Sci.,* **3**: 590-598.

ROBIN, E.D., TRAVIS, D.M., BROMBERG, P.A., FORKNER, C.E., Jr, & TYLER, J.M. (1959) Ammonia excretion by mammalian lung. *Science,* **129**: 270-271.

ROBINETTE, H.R. (1976) Effect of selected sublethal levels of ammonia on the growth of channel catfish *(Ictalurus punctatus). Prog. Fish-Cult.,* **38**(1): 26-29.

ROBINSON, E. & ROBBINS, R.C. (1968) *Sources, abundance and fate of gaseous atmospheric pollutants,* Menlo Park, Stanford Research Institute, California, 123 pp (prepared for the American Petroleum Institute).

ROBINSON, E. & ROBBINS, R.C. (1971) Emissions, concentrations, and fate of gaseous atmospheric pollutants. In: Strauss, W., ed. *Air pollution control,* New York, John Wiley and Sons, Part 2, pp. 1-93.

ROBINSON-WILSON, E.F. & SEIM, W.K. (1975) The lethal and sublethal effects of a zirconium process effluent on juvenile salmonids. *Water Resour. Bull.,* **11**(5): 975-986.

ROELOFS, J.G.M., SCHUURKES, J.A.A.R., & SMITS, A.J.M. (1984) Impact of acidification and eutrophication on macrophyte communities in soft waters in the Netherlands. II. Experimental studies. *Aquat. Bot.,* **18**: 389-411.

ROELOFS, J.G.M. (in press) The effect of sulfur and nitrogen deposition on aquatic and terrestrial heathland vegetation. *Experientia (Basel).*

ROELOFS, J.G.M., KEMPERS, A.J., HOUDYK, A.L.F.M., & JANSEN, J. (1985) The effect of airborne ammonium sulphate on *Pinus nigra var maritima,* in the Netherlands. *Plant Soil,* **84**: 45-56.

ROSAGE, T.F., SCHUTSKY, R.M., & RAPP, K.M. (1979) Toxicity of un-ionized ammonia to the spotfin shiner *(Notropis spilopterus). Proc. Pa. Acad. Sci.,* **53**: 39-42.

ROSEBOOM, D.P. & RICHEY, D.L. (1977) *Acute toxicity of residual chlorine and ammonia to some native Illinois fishes,* Urbana, Illinois, Illinois State Water Survey, 42 pp (Report of Investigation 85).

ROSENFELD, M. (1932) Experimental modification of mitosis by ammonia. *Arch. exp. Zellforsch. Gewebezuecht.,* **14**: 1.

RUBIN, A.J. & ELMARAGHY, M.A. (1976) *Studies on the toxicity of ammonia, nitrate, and their mixtures to the common guppy,* Columbus, Ohio, Water Resources Center, Ohio State University, 47 pp (Project No. A-033-OHIO).

RUBIN, A.J. & ELMARAGHY, G.A. (1977) Studies on the toxicity of ammonia, nitrate and their mixtures to guppy fry. *Water Res.,* **11**(10): 927-935.

SALVATORE, F., BOCCHINI, V., & CIMINO, F. (1963) Ammonia intoxication and its effects on brain and blood ammonia levels. *Biochem. Pharmacol.,* **12**: 1-6.

SAIFUTDINOV, M.M. (1966) Maximum permissible concentration of ammonia in the atmosphere. *Hyg. Sanit.,* **176**: 171-175.

SAMYLIN, A.F. (1969) [Effect of ammonium carbonate on early stages of development of salmon.] *Uchen. Zap. Leningr. Gos. Pedagog. Inst. Im. A. I. Gertsena,* **422**: 47-62 (in Russian).

SCHAERDEL, A.D., WHITE, W.J., LANG, C.M., DVORCHIK, B.H., & BOHNER, K. (1983) Localized and systemic effects of environmental ammonia in rats. *Lab. anim. Sci,* **33**(1): 40-45.

SCHENKER, S. & WARREN, K.S. (1962) Effect of temperature variation on toxicity and metabolism of ammonia in mice. *J. Lab. clin. Med.,* **60**: 291-301.

SCHULZE-WIEHENBRAUCK, H. (1976) Effects of sublethal ammonia concentrations on metabolism in juvenile rainbow trout (*Salmo gairdneri* Richardson). *Ber. Dtsch. Wiss. Komm. Meeresforsch.,* **24**(4): 234-250.

SCHUMAN, G.E. & BURWELL, R.E. (1974) Precipitation nitrogen contribution relative to surface runoff discharges. *J. environ. Qual.,* **3**: 366-369.

SCHUURKES, J.A.A.R. (in press) Ammonium sulfate deposition and its role in the acidification and nitrogen enrichment of poorly-buffered aquatic systems. *Experientia (Basel).*

SCHUURKES, J.A.A.R., HECK, I., HESEN, P., LEUVEN, R.S.E.W., & ROELOFS, J.G.M. (1985) Effects of acid and acidifying deposition on water composition and macrophyte composition of simulated poorly buffered aquatic systems. In: *Proceedings of the Muskoka Conference, Toronto, Canada, September 1985.*

SEEGAL, B.C. (1927) Chronic acidosis in rabbits and in dogs, with relation to kidney pathologic change. *Arch. intern. Med.,* **39**(4): 550-563.

SELESI, D. & VAMOS, R. (1976) [Factors affecting the lethal concentration of ammonia in fish ponds.] *Ichthyologia,* **8**(1): 115-121 (in Serbocroation with English summary).

SERVANT, J. & DELAPART, M. (1983) Concentrations and origins of NO_3- and NH_4+ in oceanic air. In: *CACGP Symposium on Tropospheric Chemistry with Emphasis on Sulphur and Nitrogen Cycles and the Chemistry of Clouds and Precipitation (Abstracts), 28 August - 3 September 1983, Oxford, England,* Oxford, New York, Sydney, Paris, Frankfurt, Pergamon Press,

57 pp (Fifth International Congress of the Commission on Atmoshperic Chemistry and Global Pollution).

SHAW, J. (1960) The absorption of sodium ions by the crayfish *Astacus pallipes* Lereboullet. III. The effect of other cations in the external solution. *J. exp. Biol.,* **37**(3): 548-556.

SHEN, S.S. & STEINHARDT, R.A. (1978) Direct measurement of intracellular pH during metabolic derepression of the sea urchin egg. *Nature (Lond.),* **272**(5650): 253-254.

SHERWOOD, T.K. (1925) Solubilities of sulfur dioxide and ammonia in water. *Ind. Eng. Chem.,* **17**: 745-747.

SHILO, M. & SHILO, M. (1953) Conditions which determine the efficiency of ammonium sulphate in the control of *Prymnesium parvum* in fish breeding ponds. *Appl. Microbiol.*, **1**: 330-333.

SHILO, M. & SHILO, M. (1955) Control of the phytoflagellate *Prymnesium parvum. Verh. Int. Ver. Limnol.,* **12**: 233-240.

SHOLKOVITZ, E. (1973) Interstitial water chemistry of the Santa Barbara Basin sediments. *Geochim. Cosmochim. Acta,* **37**: 2043-2073.

SHUTTLEWORTH, T.J. & FREEMAN, R.F. (1974) Factors affecting the net fluxes of ions in the isolated perfused gills of freshwater *Anguilla dieffenbachii. J. comp. Physiol.,* **94**: 297-307.

SIEGRIST, A. (1920) [Concentrated effect of alkali and acid on the eye.] *Z. Augenheilkd.,* **43**: 176-194 (in German).

SILVER, S.D. & MCGRATH, F.P. (1948) A comparison of acute toxicities of ethylene amine and ammonia to mice. *J. ind. Hyg. Toxicol.,* **30**: 7-9.

SILVERMAN, L., WHITTENBERGER, J.L., & MULLER, J. (1949) Physiological response of man to ammonia in low concentrations. *J. ind. Hyg. Toxicol.,* **31**: 74-78.

SINGER, R.H. & MCCARTY, R.T. (1971) Pathologic changes resulting from acute ammonium salt poisoning in sheep. *Am. J. Vet. Res.,* **32**: 1239-1246.

SLOAN, C.H. & MORIE, G.P. (1974) Determination of ammonia in tobacco and tobacco smoke with the ammonia electrode. *Anal. Chim. Acta,* **69**: 243-247.

SLOT, G.M.J. (1938) Ammonia gas burns: an account of six cases. *Lancet,* **2**: 1356-1357.

SMART, G. (1976) The effect of ammonia exposure on gill structure of the rainbow trout *(Salmo gairdneri). J. Fish Biol.,* **8**(6): 471-475.

SMITH, C.E. (1972) Effects of metabolic products on the quality of rainbow trout. *Am. Fishes US Trout News,* **17**(3): 7-8, 21.

SMITH, C.E. (1984) Hyperplastic lesions of the primitive meninx of fathead minnows, *Pimephales promelas,* induced by ammonia: species potential for carcinogen testing. *Natl Cancer*

Inst. Monogr., **65**: 119-125.

SMITH, C.E. & PIPER, R.G. (1975) Lesions associated with chronic exposure to ammonia. In: Ribelin, W.E. & Migaki, G., ed. *The pathology of fishes,* Madison, Wisconsin, University of Wisconsin Press, pp. 497-514.

SMITH, L.W. & WHEELER, W.E. (1979) Nutritional and economic value of animal excreta. *J. anim. Sci.,* **48**: 144.

SODERLUND, R. & SVENSSON, B.H. (1976) The global nitrogen cycle. In: Svensson, B.H. & Söderlund, R., ed. *Nitrogen, phosphorus, and sulfur: global sources,* Stockholm, pp. 23-76 (SCOPE Report No. 7) (Ecology Bulletin No. 22).

SOF'INA, E.P., VORONKOV, M.G., D'IAKOV, V.W., SOMENOVA, N.V., PLATONOVA, G.N., PERETOLCHINA, N.M., LESNAIA, N.A., & SMETANKINA, O.Z. (1978) Tris (2-oxy-ethyl) ammonium salts of some synthetic phytohormones and their antitumor activity. *Khim Farm. 2h,* **12**: 74.

SOLOMONSON, L.P. (1969) *Effects of ammonia and some of its derivatives on photosynthesis in the blue-green alga, Plectonema boryanum,* Chicago, Illinois, University of Chicago, 68 pp (Ph.D. Thesis).

SOUSA, R.J., MEADE, T.K., & FELBECK, G.T., Jr (1977) The exposure of ammonia to the photodissociated haemoglobin of the marine bloodworm, *Glycera dibranchiata. Comp. Biochem. Physiol.,* **58A**: 19-22.

SPARKS, R.E. (1975) *The acute, lethal effects of ammonia on channel catfish (Ictalurus punctatus), bluegills (Lepomis macrochirus), and fathead minnows (Pimephales promelas),* Chicago, Illinois, 15 pp (report to Illinois Institute for Environmental Quality, project No. 20.060).

SPARKS, R.E. & SANDUSKY, M.J. (1981) *Identification of factors responsible for decreased production of fish food organisms in the Illinois and Mississippi Rivers,* Havana, Illinois, River Research Laboratory, Illinois Natural History Survey, 63 pp (Project No. 3-291-R).

SPICER, C.W. (1977) The fate of nitrogen compounds in the atmosphere. In: Pitts, J.N., Jr, Metcalf, R.L., & Lloyd, A.C., ed. *Advances in environmental sciences and technology,* New York, John Wiley and Sons, Vol. 7, pp. 163-261.

SPINAZZOLA, A., MARRACCINI, L., DEVOTO, G., & ZEDDA, S. (1966) [Study of the behaviour of some volatile toxic substances as an index of air pollution in the cith of Cagliari. Note III. Ammonia.] *Folia Med. (Naples),* **49**: 641-648 (in Italian).

STAMMER, H.A. (1953) [The effect of hydrogen sulfide and ammonia on characteristic animal forms in the saprobiotic system.] *Vom Wasser,* **20**: 34-71 (in German).

STANLEY, R.A. (1974) Toxicity of heavy metals and salts to Eurasian watermilfoil *(Myriophylum spicatum). Arch. environ. Contam. Toxicol.,* **2**(4): 331-341.

STEINHARDT, R.A. & MAZIA, D. (1973) Development of K^+-conductance and membrane potentials in unfertilized sea

urchin eggs after exposure to NH_4OH. *Nature (Lond.),* **241**(5389): 400-401.

STEPHENS, E.R. (1971) Identification of odors from cattle feedlots. *Calif. Agric.,* **25**: 10-11.

STEVENS, C., KENNAWAY, N.G., & FELLMAN, J.H. (1975) Ammonia intoxicaton: A hazard during rehabilitation of protein-deprived rats. *J. Nutr.,* **i105**: 1384-1390.

STEVENSON, T.J. (1977) *The effects of ammonia, pH and salinity on the white perch, Morone americana,* Kingston, Rhode Island, University of Rhode Island, 154 pp (Ph.D. Thesis).

STEWART, B.A., VIETS, F.G., Jr, HUTCHINSON, G.L., & KEMPER, W.D. (1967) Nitrates and other water pollutants under fields and feedlots. *Environ. Sci. Technol.,* **1**(9): 736-739.

STRUZEWSKI, E.J. (1971) *Current status on control and treatment of storm and combined sewer overflow,* Edison Water Quality Laboratory (EPA Technology Transfer).

STUDIER, E.H. (1966) Studies on the mechanisms of ammonia tolerance of the guano bat. *J. exp. Zool.,* **163**: 79-85.

STUDIER, E.H., BECK, L.R., & LINDBORG, R.G. (1967) Tolerance and initial metabolic response to ammonia intoxication in selected bats and rodents. *J. Mammal.,* **48**: 564-572.

SUGIYAMA, K., YOSHIDA, M., & OYAMADA, M. (1968) [A case of chemical bronchiectasia induced by inhalation of ammonia gaz.] *Nippon Kyohbu Shikkan Gakkai Zasshi,* **27**: 797-803 (in Japanese).

SUKUMARAN, N. & KUTTY, M.N. (1977) Oxygen consumption and ammonia excretion in the catfish *Mystus armatus,* with special reference to swimming speed and ambient oxygen. *Proc. Indian Acad. Sci.,* **86B**: 195-206.

SUMMERSKILL, W.H.J. & WOLPERT, E. (1970) Ammonia metabolism in the gut. *Am. J. clin. Nutr.,* **23**: 633-639.

SUYAMA, M., KOIKE, J., & SUZUKI, K. (1960) Studies on the glycolysis and the formation of ammonia in the muscle and blood of elasmobranchs. *J. Tokyo Univ. Fish.,* **46**: 51-60.

SWIGERT, J.P. & SPACIE, A. (1983) *Survival and growth of warmwater fishes exposed to ammonia under low flow conditions,* Springfield, Virginia, National Technical Information Service (NTIS PB83-257535).

TABATA, K. (1962) [Toxicity of ammonia to aquatic animals with reference to the effect of pH and carbon dioxide.] *Hokoku,* **34**: 67-74 (in Japanese).

TAIGANIDES, E.P. & WHITE, R.K. (1969) The menace of noxious gases in animal units. *Trans. Am. Soc. Agric. Eng.,* **12**: 359-362.

TAKAHASHI, H., WAKAI, K., & OHUCHI, K. (1984) [Experiences of chemical burns induced by ammonia gaz.] *Nippon Saigai Igakkai Kaishi,* **32**: 741-749 (in Japanese).

TAPLIN, G.V., CHOPRA, S., YANDA, R., & ELAM, D. (1976) Radionuclidic lung-imaging procedures in the assessment of

injury due to ammonia inhalation. *Chest,* **69**: 5.

TAYLOR, A.W., EDWARDS, W.M., & SIMPSON, E.C. (1971) Nutrients in streams draining woodland and farmland near Coshocton, Ohio. *Water Resour. Res.,* **7**: 81-89.

TAYLOR, J.E. (1973) Water quality and bioassay study from Crawford National Fish Hatchery. *Trans. Nebr. Acad. Sci.,* **2**: 176-181.

THE NETHERLANDS MINISTRY OF HOUSING, PHYSICAL PLANNING AND ENVIRONMENT (1984) Acidification in the Netherlands: effects and policies. In: *Proceedings of a Conference on Air Pollution in Europe, Munich, 24-27 June, 1984,* The Netherlands Ministry of Housing, Physical Planning and Environment, pp. 9.

THOMAS, W.H., HASTINGS, J., & FUJITA, M. (1980) Ammonium input to the sea via large sewage outfalls. Part 2. Effects of ammonium on growth and photosynthesis of southern California phytoplankton cultures. *Mar. environ. Res.,* **3**(4): 291-296.

THOMPSON, R.Y. & HALLIBURTON, I.W. (1966) Effect of diet on the composition of the kidney. *Biochem. J.,* **99**(3): 44.

THORNTON, N.C. & SETTERSTROM, C. (1940) Toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide, and sulphur dioxide gases. III. Green plants. *Contrib. Boyce Thompson Inst.,* **11**: 343-356.

THURSTON, R.V. (1981) *Acute toxicity of ammonia to the pumpkinseed (Lepomis gibbosus),* Duluth, Minnesota, US Environmental Protection Agency, Environmental Research Laboratory.

THURSTON, R.V. & MEYN, E.L. (1984) *Acute toxicity of ammonia to five fish species from the northwest United States,* Bozeman, Montana Fisheries Bioassay Laboratory, Montana State University, 13 pp (Technical Report Vol. 84, No. 4).

THURSTON, R.V. & RUSSO, R.C. (1981) *Acute toxicity of ammonia to golden trout (Salmo aguabonita) and mottled sculpin (Cottus bairdi),* Bozeman, Montana, Fisheries Bioassay Laboratory, Montana State University, 10 pp (Technical Report Vol. 81, No. 1).

THURSTON, R.V. & RUSSO, R.C. (1983) Acute toxicity of ammonia to rainbow trout. *Trans. Am. Fish. Soc.,* **112**: 696-704.

THURSTON, R.V., RUSSO, R.C., & SMITH, C.E. (1978) Acute toxicity of ammonia and nitrite to cutthroat trout fry. *Trans. Am. Fish. Soc.,* **107**(2): 361-368.

THURSTON, R.V., CHAKOUMAKOS, C., & RUSSO, R.C. (1981a) Effect of fluctuating exposures on the acute toxicity of ammonia to rainbow trout *(Salmo gairdneri)* and cutthroat trout *(S. clarki). Water Res.,* **15**(7): 911-917.

THURSTON, R.V., PHILLIPS, G.R., RUSSO, R.C., & HINKINS, S.M. (1981b) Increased toxicity of ammonia to rainbow trout *(Salmo gairdneri)* resulting from reduced concentrations of dissolved oxygen. *Can. J. Fish. aquat. Sci.,* **38**(8): 983-988.

THURSTON, R.V., RUSSO, R.C., & VINOGRADOV, G.A. (1981c) Ammonia toxicity to fishes: Effect of pH on the toxicity of

the un-ionized ammonia species. *Environ. Sci. Technol.,* **15**(7): 837-840.

THURSTON, R.V., RUSSO, R.C., & PHILLIPS, G.R. (1983) Acute toxicity of ammonia to fathead minnows. *Trans. Am. Fish. Soc.,* **112**: 705-711.

THURSTON, R.V., LUEDTKE, R.J., & RUSSO, R.C. (1984a) *Toxicity of ammonia to fresh-water insects of three families,* Bozeman, Montana, Fisheries Bioassay Laboratory, Montana State University, 26 pp (Technical Report Vol. 84, No. 2).

THURSTON, R.V., RUSSO, R.C., LUEDTKE, R.J., SMITH, C.E., MEYN, E.L., CHAKOUMAKOS, C., WANG, K.C., & BROWN, C.J.D. (1984b) Chronic toxicity of ammonia to rainbow trout. *Trans. Am. Fish. Soc.,* **113**(1): 56-73.

THURSTON, R.V., MEYN, E.L., ZAJDEL, R.K., & RUSSO, R.C. (submitted) Chronic toxicity of ammonia to fathead minnows *(Pimephales promelas).* Submitted to Trans. Am. Fish. Soc.

TMRI (1971) [Atmosphere division. On survey of air pollutants peculiar to factory-clustered area.] *Tokyo Meteorol. Res. Inst. Environ. Prot.,* **2**: 109-111 (Annual Report) (in Japanese).

TOMASSO, J.R., GOUDIE, C.A., SIMCO, B.A., & DAVIS, K.B. (1980) Effects of environmental pH and calcium on ammonia toxicity in channel catfish. *Trans. Am. Fish. Soc.,* **109**(2): 229-234.

TOPPING, D.C. & VISEK, W.J. (1976) Nitrogen intake and tumourigenesis in rats injected with 1,2-dimethylhydrazine. *J. Nutr.,* **106**: 1583.

TORDA, C. (1953) Ammonium ion content and electrical activity of the brain during the preconvulsive and convulsive phases induced by various convulsants. *J. Pharmacol. exp. Ther.,* **107**: 197-203.

TOTH, B. (1972) Hydrazine, methylhydrazine, and methylhydrazine sulfate carcinogenesis in Swiss mice. Failure of ammonium hydroxice to interfere in the development of tumors. *Int. J. Cancer,* **9**: 109.

TOWLE, D.W., PALMER, G.E., & HARRIS, J.L. III (1976) Role of gill $Na^+ + K^+$-dependent ATPase in acclimation of blue crabs *(Callinectes sapidus)* to low salinity. *J. exp. Biol.,* **196**: 315-322.

TOWLE, D.W. & TAYLOR, D.D. (1976) Effect of NH_4^+ and K^+ on Na^+-transport ATPase activity of blue crab gill *Am. Zool.,* **16**: 224.

TRESHOW, M. (1970) Ammonia. In: *Environment and plant response,* New York, McGraw-Hill Book Company, pp. 362-363.

TURNBULL, H., DEMANN, J.G., & WESTON, R.F. (1954) Toxicity of various refinery materials to fresh water fish. *Ind. Eng. Chem.,* **46**(2): 324-333.

ULRICH, B. (1983) Soil acidity and its relation to acid deposition. In: *Effects of accumulation of air pollutants in forest ecosystems,* Ulrich and Pankrasth, The Netherlands,

D. Reidel Publishing Company, pp. 127-146.

ULSHAFER, T.R. (1958) The measurement of changes in acetylcholine level (ACh) in rat brain following ammonium ion intoxication and its possible bearing on the problem of hepatic coma. *J. lab. clin. Med.,* **52**: 718-723.

ULTSCH, G.R., JACKSON, D.C., & MOALLI, R. (1981) Metabolic oxygen conformity among lower vertebrates: the toadfish revisited. *J. comp. Physiol.,* **142**: 439-443.

UNDERWRITERS LABORATORIES (1933) *Report on the comparative life, fire, and explosion hazards of common refrigerants. Miscellaneous Hazard No. 2375,* Chicago, Illinois, Underwriters Laboratories, pp. 26-28.

UNITED KINGDOM MINISTRY OF TECHNOLOGY (1963) *Effects of pollution on fish; toxicity of mixutures of zinc, sulfate, and ammonium chloride; toxicity of sewage effluents; the toxicity of poisons under estuarine conditions,* London, United Kingdom Ministry of Technology, pp. 76-83 (Water Pollution Research 1962).

UNITED KINGDOM MINISTRY OF TECHNOLOGY (1968) *Effects of pollution on fish,* London, United Kingdom Ministry of Technology, pp. 56-65 (Water Pollution Research 1967).

US EPA (1972) *Air quality data for 1968 from the National Air Surveillance Networks and contributing state and local networks,* Research Triangle Park, North Carolina, US Environmental Protection Agency, Office of Air Programs, 231 pp (Report No. APTD-0978).

US EPA (1979a) *Ammonia study using STORET ambient monitoring data,* Washington DC, US Environmental Protection Agency, Monitoring and Data Support Division, Office of Water Planning and Standards.

US EPA (1979b) *Methods for chemical analysis of water and wastes,* Cincinnati, Ohio, US Environmental Protection Agency, Environmental Monitoring and Support Laboratory (Report No. EPA 600/4-79-020).

US EPA (1981) *Multimedia health assessment document on ammonia,* Cincinnati, Ohio, US Environmental Protection Agency, Environmental Criteria and Assessment Office (Report No. ECAO/82-DO10).

US EPA (1985) *Ambient water quality criteria for ammonia - 1984,* Duluth, Minnesota, US Environmental Protection Agency, Office of Research and Development.

US NIOSH (1974) *Criteria for a recommended standard. Occupational exposure to ammonia,* Washington DC, National Institute for Occupational Safety and Health, pp. 74-136 (US DHEW Publication No. 246-699).

US NIOSH (1977) *NIOSH manual of analytical methods. Part I. NIOSH monitoring methods,* Washington DC, National Institute for Occupational Safety and Health, Vol. 1, pp. 205.1-205.3 (US DHEW Publication No. 77-157-A).

UZVOLGYI, E. & BOJAN, F. (1980) Possible *in vivo* formation of a carcinogenic substance from diethyl pyrocarbonate and

ammonia. *J. Cancer Res. clin. Oncol.,* **97**: 205.

VALENTINE, H. (1964) A study of the effect of different ventilation rates on the ammonia concentrations in the atmosphere of broiler houses. *Br. Poult. Sci.,* **5**: 149-159.

VAMOS, R. (1963) Ammonia poisoning in carp. *Acta. biol. Szeged.,* **9**(1-4): 291-297.

VAMOS, R. & TASNADI, R. (1967) Ammonia poisoning in carp. 3. The oxygen content as a factor influencing the toxic limit of ammonia. *Acta. biol. Szeged.,* **13**(3-4): 99-105.

VAN AALST, R.M. (1984) *[Atmospheric process and deposition,]* Ministry of Agriculture and Fisheries, Ministry of Housing, Physical Planning and Environment, 76 pp (Report No. 84(2)) (in Dutch).

VAN BREEMEN, N. & JORDENS, E.R. (1983) Effects of atmospheric ammonium sulfate on calcareous and non-calcareous soils of woodlands in the Netherlands. In: Ulrich, B. & Panlerath, J., ed. *Effects of accumulation of air pollutants in forest ecosystems,* The Netherlands, Reidel Publishing Company, pp. 171-182.

VANCURA, E.M., CLINTON, J.E., RUIZ, E., & KRENZELOK, E.P. (1980) Toxicity of alkaline solutions. *Ann. emergency Med.,* **9**(3): 118-122.

VAN DER EERDEN, L.J.M. (1982) Toxicity of ammonia to plants. *Agric. Environ.,* **7**: 223-235

VAN SLYKE, D.D., PHILLIPS, R.A., HAMILTON, P.B., ARCHIBALD, R.M., FUTCHER, P.H., & MILLER, A. (1943) Glutamine as source material of urinary ammonia. *J. Biol. Chem.,* **150**: 481-482.

VAUGHN, R.E. & SIMCO, B.A. (1977) Effects of ammonia on channel catfish, *Ictalurus punctatus. ASB Bull.,* **24**(2): 92.

VENKATARAMIAK, A., LAKSHMI, G.J., BEST, C., GUNTER, G., HARTWIG, E., & WILDE, P. (1981) *Studies on toxicity of OTEC plant components on marine animals from the Gulf of Mexico,* Springfield, Virginia, National Technical Information Service (DE81030167).

VERBERK, M.M. (1977) Effects of ammonia in volunteers. *Int. Arch. occup. environ. Health,* **39**: 73 -81.

VIGLIANI, E.C. & ZURLO, N. (1955) [Experiences of the Clinica del Lavoro with their own maximal exposure limits for industrial chemicals.] *Arch. Gewerbepathol. Gewerbehyg.,* **13**: 528-534 (in German).

VINES, H.M. & WEDDING, R.T. (1960) Some effects of ammonia on plant metabolism and a possible mechanism for ammonia toxicity. *Plant Physiol.,* **35**: 820-825.

VINOKUROVA, M.K. & MAL'KOVA, V.B. (1963) [Toxicology of the selective herbicide ammonium sulfamate.] *Gig. Trud. Prof. Zabol.,* **7**(5): 56-57 (in Russian).

VISEK, W.J., KOLONDY, G.M., & GROSS, P.R. (1972) Ammonia effects in cultures of normal and transformed 3T3 cells. *J. cell Physiol.,* **80**: 373.

VISEK, W.J., CLINTON, S.K., & TRUEX, C.R. (1978) Nutrition and experimental carcinogenesis. *Cornell Vet.,* **68**: 3.

VITTI, T.G., VUKMIROVICH, R., & GAEBLER, O.H. (1964) Utilization of ammonia nitrogen, administered by intragastric, intraperitoneal and subcutaneous roues: Effects of growth hormone. *Arch. Biochem. Biophys.,* **106**: 475-482.

WALLEN, I.E., GREER, W.C., & LASATER, R. (1957) Toxicity to *Gambusia affinis* of certain pure chemicals in turbid waters. *Sewage Ind. Wastes,* **29**(6): 695-711.

WALLINGFORD, G.W. (1977) Fate of nitrogen from fertilizer practices. In: *Disposal of residues on land,* Rockville, Maryland, Information Transfer, Inc., pp. 152-155.

WALSH, L.M. (1977) Updating the nitrogen cycle. In: *Disposal of residues on land,* Rockville, Maryland, Information Transfer, Inc., pp. 146-151.

WALTON, M.J. & COWEY, C.B. (1977) Aspects of ammoniogenesis in rainbow trout, *Salmo gairdneri. Comp. Biochem. Physiol.,* **57B**: 143-149.

WALUGA, D. & FLIS, J. (1971) [Changes in peripheral blood in carp (*Cyprinus carpio* L.) under the influence of ammonia solution.] *Rocz. Nauk Roln. Ser. H,* **93**(2): 87-94 (in Russian).

WARNCKE, D.D. & BARBER, S.A. (1973) Ammonium and nitrate uptake by corn (*Zea mays* L.) as influenced by nitrogen concentration and NH_4-/NO_3-ratio. *Agric. J.,* **65**: 950-953.

WARREN, K.S. (1958) The differential toxicity of ammonium salts. *J. clin. Invest.,* **37**: 497-501.

WARREN, K.S. & NATHAN, D.G. (1958) The passage of ammonia across the blood, brain, barrier and its relation to blood pH. *J. clin. Invest.,* **37**: 1724-1728.

WARREN, K.S. & SCHENKER, S. (1964) Effect of an inhibitor of glutamine synthesis (methionine sulfoximine) on ammonia toxicity and metabolism. *J. lab. clin. Med.,* **64**: 442-449.

WARREN SPRING LABORATORY (1982) *Acidity of rainfall in the United Kingdom.*

WATTS, R.L. & WATTS, D.C. (1974) Nitrogen metabolism in fishes. In: Florkin, M. & Scheer, B.T. ed. *Chemical Zoology,* New York, Academic Press, Vol. III, pp. 369-446.

WEAST, R.C., ed. (1979) *CRC handbook on chemistry and physics,* 59th ed., Boca Raton, Florida, CRC Press Inc.

WEATHERBY, J.H. (1952) Chronic toxicity of ammonia fumes by inhalation. *Proc. Soc. Exp. Biol. Med.,* **81**: 300-301.

WEBB, D.W., BARTLEY, E.E., & MEYER, R.M. (1972) A comparison of nitrogen metabolism and ammonia toxicity from ammonium acetate and urea in cattle. *J. anim. Sci.,* **35**: 1263-1270.

WEBB, J.T. & BROWN, G.W., Jr (1976) Some properties and occurrence of glutamine synthetase in fish. *Comp. Biochem. Physiol.,* **54B**: 171-175.

WEDDING, R.T. & VINES, H.M. (1959) Inhibition of reduced diphosphopyridine nucleotide oxidation by ammonia. *Nature (Lond.),* **184**: 1226-1227.

WEEKS, M.E. (1968) *Discovery of the elements,* 7th ed., Washington DC, Journal of Chemical Education, p. 197.

WEHRBEIN, M.F., VIPPERMAN, P.E., Jr, PEO, E.R., Jr, & CUNNINGHAM, P.J. (1970) Diammonium citrate and diammonium phosphate as sources of dietary nitrogen for growing-finishing swine. *J. anim. Sci.,* **31**: 327-332.

WHITE, A., HANDLER, P., & SMITH, E.L. (1973) Renal function and the composition of urine. In: *Principles of biochemistry,* 5th ed., New York, McGraw Hill Book Co., pp. 604-704 and 928-948.

WHO (1966) *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases. Ninth Report of the Expert Committee,* Geneva, World Health Organization (WHO Technical Report Series No. 339).

WHO (1974a) *Toxicological evaluation of certain food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents,* Geneva, World Health Organization (WHO Food Additives Series No. 5).

WHO (1974b) *Toxicological evaluation of certain food additives with a review of general principles and of specification. 17th report of the Expert Committee,* Geneva, World Health Organization (WHO Technical Report Series No. 539).

WHO (1980) *Evaluation of certain food additives. Twenty-third Report of the Expert Committee,* Geneva, World Health Organization (WHO Technical Report Series No. 648).

WHO (1982a) *Evaluation of certain food additives and contaminants. Twenty-sixth Report of the Expert Committee,* Geneva, World Health Organization (WHO Technical Report Series No. 683).

WHO (1982b) *Toxicological evaluation of certain food additives,* Geneva, World Health Organization (WHO Food Additives Series No. 17).

WICKINS, J.F. (1976) The tolerance of warm-water prawns to recirculated water. *Aquaculture,* **9**(1): 19-37.

WILKIN, D.C. & FLEMAL, R.C. (1980) Feasibility of water quality improvement in three Illinois rivers. *J. Water Poll. Control Fed.,* **52**(2): 293.

WILKINSON, R.H., THOMAS, W.J., HANSEN, C.M., & SHIELDS, E.A. (1978) *Forage preservation by timed-slow release of* NH_3 (ASAE Paper No. 78-1523).

WILSON, R.P., DAVIS, L.E., HUMRER, M.E., & BLOOMFIELD, R.A. (1968a) Toxicologic effects of ammonium carbamate and related compounds. *Am. J. vet. Res.,* **29**: 987-906.

WILSON, R.P., DAVIS, L.E., HUMRER, M.E., & BLOOMFIELD, R.A. (1968b) Comparative ammonia toxicity. *Comp. Biochem. Physiol.,* **25**: 295-301.

WILT, F.H. & MAZIA, D. (1974) The stimulation of cytoplasmic polyadenylyation in sea urchin eggs by ammonia. *Dev. Biol.,* **37**: 422-424.

WINDHOLZ, M., BUDAVARI, S., STROUMTSOS, L.Y., & FERTIG, M.N., ed. (1976) *Merck Index,* 9th ed., Rahway, New Jersey, Merck and Co. Inc., p. 69.

WINKLER, M.M. & GRAINGER, J.L. (1978) Mechanisms of action of NH_4Cl and other weak bases in the activation of sea urchin eggs. *Nature (Lond.),* **273**(5663): 536-538.

WOKER, H. & WUHRMANN, K. (1950) [The sensitivity of different species of fish to ammonia, hydrocyanic acid and phenol.] *Rev. Suisse Zool.,* **57**(3): 548-553 (in German).

WOLAVER, T.C. (1972) *Distribution of natural and anthropogenic elements and levels in precipitation across the US: theory and quantitative models,* Chapel Hill, North Carolina, University of North Carolina, 75 pp (Prepared for US EPA).

WOOD, R.W. (1979) Behavioral evaluation of sensory irritation evoked by ammonia. *Toxicol. appl. Pharmacol.,* **50**: 157-162.

WORCEL, A. & ERECINSKA, M. (1962) Mechanism of inhibitory action of ammonia on the respiration of rat liver mitochondria. *Biochim. Biophys. Acta,* **65**: 27-33.

WRIGHT, P.A. & WOOD, C.M. (1985) An analysis of branchial ammonia excretion in the freshwater rainbow trout: effects of environmental pH change and sodium uptake blockage. *J. exp. Biol.,* **114**: 329-353.

WUHRMANN, K. & WOKER, H. (1948) [Contributions to the toxicology of fishes. II. Experimental investigations on ammonia and hydrocyanic acid poisoning.] *Schweiz. Z. Hydrol.,* **11**: 210-244 (in German).

WUHRMANN, K., ZEHENDER, F., & WOKER, H. (1947) [Biological significance for fisheries of ammonium-ion and ammonia content of flowing bodies of water.] *Vierteljahrsschr. Naturforsch. Ges. Zurich,* **92**: 198-204 (in German).

YAHAGI, H., SUZUKI, K., & MATSUI, Y. (1959) [Fourteen cases of ammonia gas poisoning.] *Shuhkan Igaku Tsuhshin,* **601**: 26-30 (in Japanese).

YECK, R.G., SMITH, L.W., & CALVERT, C.C. (1975) Recovery of nutrients from animal wastes. An overview of existing options and potentials for use in feed. In: *Managing livestock waste: Proceedings of the Third International Symposium on Livestock Wastes,* St. Joseph, Michigan, American Society of Agricultural Engineers, pp. 192-196.

YOSHIDA, J., NAKAME, K., & NAKAMURA, R. (1957) Toxicity of urea and its control. II. Toxicity of ammonium salts and urea in rabbits and goats. *Nippon Chikusangaku Kaiho,* **28**: 185-191.

ZIEVE, L., DOIZAKI, W.M., & ZIEVC, F.J. (1974) Synergism between mercaptans and ammonia or fatty acids in the production of coma. A possible role for mercaptans in the pathogenesis of hepatic coma. *J. lab. clin. Med.,* **83**: 16-28.

ZIMBER, A. (1970) *Studies on the effect of ammonia on DNA synthesis and on cellular proliferation and renewal in tissues of the mouse and in Ehrlich ascites tumor cells*, Ithaca, New York, Cornell University (Ph.D. Thesis).

ZIMBER, A. & VISEK, W.J. (1972) Effect of urease injection on DNA synthesis in mice. *Am. J. Physiol.,* **223**: 1004.

ZIMMERMAN, P.W. (1949) Impurities in the air and their influence on plant life. In: *Proceedings of the First National Air Pollution Symposium,* Pasadena, California, 10-11 November, 1949, pp. 135-141 (Sponsored by Stanford Research Institute).

ZLATEVA, M., VALCEVA, V., & ANTOV, G. (1974) Toxic organic changes in laboratory animals placed in an ammonia-rich working atmosphere. *Tr. Inst. Hig. Ohrana Tr. Prof. Zabol.,* **22**(1): 147-154.

Annex I. Ammonium salts evaluations prepared by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[a]

Ammonium salt	Functional use	Evaluation status (ADI, MTDI)[b,c]
acetate	pH-adjusting agent	not specified[d] (grouped wit other ammonium salts and a
alginate	thickening agent, stabilizer	0 - 50 (grouped with algin and calcium, potassium, an sodium alginate)
bicarbonate (hydrogen carbonate) carbonate	leavening agent, buffer neutralizing agent, alkali	not specified
chloride	dough conditioner, yeast, food	not specified (grouped wit hydrochloric acid and magn and potassium chlorides)
hydrogen phosphate phosphate dibasic	buffering agent, dough conditioner, leavening agent, yeast	MTDI:, 70 expressed as pho (grouped with phosphates a polyphosphates, including phosphates occurring natur food)
hydroxide (strong ammonia solution)	alkali	not specified
lactate	buffer, dough conditioner	not specified
persulfate	flour-treatment agent	no ADI set

[a] Queries concerning updated information should be addressed to: Joint WHO Secret FAO/WHO Expert Committee on Food Additives, International Programme on Chemica Organization, Geneva, Switzerland.

[b] ADI = Acceptable daily intake for man expressed as mg/kg body weight.

[c] MTDI = Maximum tolerable daily intake for man, expressed as mg/kg body weight.

[d] ADI not specified = the total intake of the substance arising from its uses at to achieve the desired effect does not represent a hazard to health.

REFERENCES TO ANNEX I

WHO (1966) *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour-treatment agents, acids, and bases. Ninth Report of the Expert Committee,* Geneva, World Health Organization (WHO Technical Report Series No. 339).

WHO (1974a) *Toxicological evaluation of certain food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents,* Geneva, World Health Organization (WHO Food Additives Series No. 5).

WHO (1974b) *Toxicological evaluation of certain food additives with a review of general principles and of specification. 17th report of the Expert Committee,* Geneva, World Health Organization (WHO Technical Report Series No. 539).

WHO (1980) *Evaluation of certain food additives. Twenty-third Report of the Expert Committee,* Geneva, World Health Organization (WHO Technical Report Series No. 648).

WHO (1982a) *Evaluation of certain food additives and contaminants. Twenty-sixth Report of the Expert Committee,* Geneva, World Health Organization (WHO Technical Report Series No. 683).

WHO (1982b) *Toxicological evaluation of certain food additives,* Geneva, World Health Organization (WHO Food Additives Series No. 17).

ANNEX II: TREATMENT OF EXCESSIVE EXPOSURE TO AMMONIA

Ammonia (gas and liquid) is an extremely irritant chemical affecting the skin, eyes, and the respiratory tracts. Ammonia gas can produce burning of the eyes, lachrymation, and severe eye damage. When inhaled it can produce coughing, laryngitis, bronchitis, chest pains, and severe respiratory problems. Contact with liquid ammonia can result in severe eye and skin burns due both to its irritant properties and chilling effect.

Those working with ammonia should be trained in its safe use including the dangers of improper handling, the use of protective equipment, and the avoidance of unnecessary inhalation of the gas and direct contact with liquid ammonia. After handling liquid ammonia, the hands should be washed thoroughly before eating or smoking.

The provision of protective clothing and equipment is not an adequate substitute for safe working conditions. However, where exposure cannot be adequately controlled, workers should be provided with suitable impervious clothing, boots and gloves, and, depending on the severity of the conditions, a face shield or safety goggles and a mask or self contained breathing apparatus. In places where very high gaseous ammonia concentrations are expected, complete gas suits should be used.

Emergency showers and eye wash or water sprays should be provided in all areas where ammonia is handled and where leaks, spills or splashes may occur. Clothes contaminated with ammonia

should be discarded immediately and not worn again until thoroughly cleansed.

First aid

If excessive exposure has occurred first aid treatment should be promptly initiated and medical advice obtained as soon as possible.

Ammonia in the eye (gas, liquid, or liquor)

Ammonia in the eye may cause severe injury and must be treated immediately by irrigation for at least 15 min with flowing water or sterile buffered eye irrigation solution.

Ammonia on the skin (liquid or liquor)

Drench the affected area with water and remove contaminated clothing and footwear. Wash the affected area continuously for 5 - 10 min or until pain ceases.

Ingestion (liquid or liquor)

If the patient is conscious large quantities of water may be given to dilute the chemical in the stomach. No attempt should be made to induce vomiting.

Inhalation of gas/vapour

1. Remove from exposure, secure airway and place in semiprone recovery position if unconscious. Give artificial respiration if not breathing.

2. If heartbeat is absent give external cardiac massage.

3. If there is cyanosis (blueness of lips) or air-hunger administer oxygen by facemask.

4. A conscious patient may be given water to drink.

Further treatment

Ammonia in the eyes

Corneal damage is probable. Use local anaesthetics and cycloplegics to enable thorough irrigation and examination. If the cornea is damaged, administer topical antibiotics. Refer to a specialist centre.

Ammonia on the skin

Treat as a chemical burn. Liquid ammonia may produce deep burns that may require grafting. Refer deep or extensive burns to a specialist centre.

Inhalation of gas/vapour

1. Ammonia is irritant to the respiratory tract causing:

   (a) bronchial oedema, spasm, and hypersecretion resulting in chest tightness, wheeze, and cough, which may progress to severe dyspnoea; and

   (b) lower airway inflammation with exudative

pulmonary oedema and impaired gaseous diffusion. Symptoms may be delayed 24 h or more. Resolution may be by fibrosis producing a restrictive defect.

2. Treat hypoxia with oxygen, ventilation, and bronchial lavage, as appropriate.

3. Consider administration of steroids by multiple metered doses of topical aerosol, by inhalation, and/or by injection. Early prophylactic use may be indicated.

4. Administer bronchodilators by inhalation or injection, as indicated. Maintain with oral treatment.

5. Keep under medical surveillance for at least 48 h. Treat symptomatically.

6. Observe for secondary respiratory infection and treat as necessary.

See Also:
Toxicological Abbreviations
Ammonia (HSG 37, 1990)