FOREWORD INTRODUCTION

AMMONIUM SULFATE

CAS N°: 7783-20-2

SIDS Initial Assessment Report

For

SIAM 19

Berlin, Germany, 19–22 October 2004

1.	**Chemical Name:**	Ammonium Sulfate
2.	**CAS Number:**	7783-20-2
3.	**Sponsor Country:**	Germany Contact Point: BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit) Contact person: Prof. Dr. Ulrich Schlottmann Postfach 12 06 29 D- 53048 Bonn-Bad Godesberg
4.	**Shared Partnership with:**	BASF AG/Germany;
5.	**Roles/Responsibilities of the Partners:**	-
•	Name of industry sponsor /consortium	BASF AG/Germany Contact: Dr. Rolf Sarafin, GUP/CL - Z570 Carl-Bosch-Strasse D-67056 Ludwigshafen on behalf of the Ammonium sulfate consortium
•	Process used	see next page
6.	**Sponsorship History**	
•	How was the chemical or category brought into the OECD HPV Chemicals Program?	By ICCA initiative
7.	**Review Process Prior to the SIAM:**	last literature search (update): 29 September 2003 (Human Health): databases medline, toxline; search profile CAS-No. and special search terms 25 August 2003 (Ecotoxicology): databases CA, biosis; search profile CAS-No. and special search terms OECD/ICCA
8.	**Quality check process:**	IUCLID was used as a basis for the SIDS dossier. All data were checked and validated by BUA. A final evaluation of the human health part has been performed by the Federal Institute for Risk Assessment (BfR) and of the ecotoxicological part by the Federal Environment Agency (UBA).
9.	**Date of Submission:**	Deadline for circulation: 23 July 2004

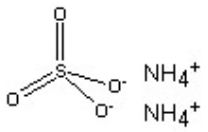
10. Comments: -

OECD/ICCA - The BUA* Peer Review Process

Qualified BUA personnel (toxicologists, ecotoxicologists) perform a quality control on the full SIDS dossier submitted by industry. This quality control process follows internal BUA guidelines/instructions for the OECD/ICCA peer review process and includes:
– a full (or update) literature search to verify completeness of data provided by industry in the IUCLID/HEDSET
– Review of data and assessment of the quality of data
– Review of data evaluation
– Check of adequacy of selection process for key studies for OECD endpoints, and, where relevant, for non-OECD endpoints by checking original reports/publications
– Review of key study description according robust summaries requirements; completeness and correctness is checked against original reports/publications (if original reports are missing: reliability (4), i.e. reliability not assignable)
– Review o f validity of structure-activity relationships
– Review of full SIDS dossier (including SIAR, SIAP and proposal for conclusion and recommendation for further work)
– In case of data gaps, review of testing plan or rationale for not testing

* BUA (GDCh-Beratergremium für Altstoffe): Advisory Committee on Existing Chemicals of the Association of German Chemists (GDCh)

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	7783-20-2
Chemical Name	Ammonium sulfate
Structural Formula	$(NH_4)_2SO_4$ 

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

Fertility and developmental toxicity studies with ammonium sulfate were not available. As ammonium sulfate dissociates in biological systems studies with other ammonium and sulfate salts can be used to cover these endpoints: A screening study according to OECD TG 422 with ammonium phosphate as analogue substance, which forms ammonium ions in aqueous solutions is available. Fully valid fertility studies with analogue compounds containing sulfate ions are however lacking. Two limited studies with sodium sulfate can be used for assessment of fertility and developmental toxicity, however, in none of these studies have the fetuses been examined histologically. There are no in vivo data on genotoxicity for ammonium sulfate. To bridge the data gap, data for ammonium chloride, which dissociates in aqueous media to form ammonium ions, as does ammonium sulfate, will be used.

In aqueous media, ammonium sulfate dissociates in the ammonium and sulfate ions (NH_4^+, SO_4^{2-}). These can be taken up into the body by the oral and respiratory routes. Absorbed ammonium is transported to the liver and there metabolised to urea and excreted via the kidneys. Ammonium is also an endogenous substance that serves a major role in the maintenance of the acid-base balance. Minor amounts of ammonium nitrogen are incorporated in the physiological N-pool. Sulfate is a normal intermediate in the metabolism of endogenous sulfur compounds, and is excreted unchanged or in conjugated form in urine.

Ammonium sulfate is of relatively low acute toxicity (LD_{50}, oral, rat: 2000 - 4250 mg/kg bw; LD_{50} dermal, rat/mouse > 2000 mg/kg bw; 8-h LC_{50}, inhalation, rat > 1000 mg/m^3). Clinical signs after oral exposure included staggering, prostration, apathy, and laboured and irregular breathing immediately after dosing at doses near to or exceeding the LD_{50} value. In humans, inhalation exposure to 0.1 – 0.5 mg ammonium sulfate/m³ aerosol for two to four hours produced no pulmonary effects. At 1 mg ammonium sulfate/m^3 very slight pulmonary effects in the form of a decrease in expiratory flow, in pulmonary flow resistance and dynamic lung compliance were found in healthy volunteers after acute exposure.

Neat ammonium sulfate was not irritating to the skin and eyes of rabbits. There is no data on sensitisation available.

A 14-day inhalation study on rats exposed to 300 mg/m^3 , the only tested dose, did not report histopathological changes in the lower respiratory tract. As the respiratory tract is the target organ for inhalation exposure, the NOEL for toxicity to the lower respiratory tract is 300 mg/m^3.
The NOAEL after feeding diets containing ammonium sulfate for 13 weeks to rats was 886 mg/kg bw/day. The only toxicity sign found was diarrhea in male animals of the high-dose group (LOAEL: 1792 mg/kg bw/day).

Ammonium sulfate was not mutagenic in bacteria (Ames test) and yeasts with and without metabolic activation systems. It did not induce chromosomal aberrations in mammalian or human cell cultures. No in vivo genotoxicity tests are available. Based on the negative results from in vitro studies and the negative results in the micronucleus test in vivo with ammonium chloride a mutagenic activity of ammonium sulfate in vivo is unlikely.

Similarly to other salts, high doses of ammonium sulfate may have the capability of tumor promotion in the rat stomach; it is, however, much less potent than sodium chloride when tested under identical conditions.

There are no valid studies available on the effects of ammonium sulfate on fertility and development. Based on data from a similar ammonium compound (diammonium phosphate), which has been tested up to 1500 mg/kg bw in a screening study according to OECD TG 422 in rats it can be concluded that ammonium ions up to the dose tested have no negative effects on fertility. In the 13-week feeding study of ammonium sulfate with rats, no histological changes of testes were observed up to 1792 mg/kg bw. The ovaries were not examined. Fully valid studies with sulfate on fertility are not available.

In a limited study (pretreatment time short, low number of animals, no fertility indices measured) where female mice were treated with up to ca. 6550 mg sulfate/kg bw (as sodium sulfate) no effects on litter size were found.

Studies of developmental toxicity for ammonium sulfate are not available. In the screening study according to OECD TG 422 with up to 1500 mg diammonium phosphate/kg bw no effects on development have been detected in rats. In another limited screening study with exposure of mice to a single dose of 2800 mg sodium sulfate/kg bw no macroscopic effects or adverse effects on body weight gain have been detected in the pups. In both studies fetuses were not examined histopathologically.

Environment

Ammonium sulfate is a white solid, with a solubility in water of 764 g/l at 25 °C. When heated, decomposition starts at temperatures between 150 and 280 °C, depending on the experimental conditions and purity of the test substance, and is complete at 336 - 357 °C. The relative density is 1.77, and the partial pressure of ammonia over solid ammonium sulfate at 25 °C is $4.053*10^{-7}$ Pa. The log Kow was determined as –5.1 in a test according to OECD TG 107; as this method applies only to substances which do not dissociate, the validity of this method for ammonium sulfate is uncertain. Due to the ionic nature of the substance the calculation of sorption onto organic soil matter does not have any practical meaning.

Due to the salt-character of the substance the calculation of a fugacity model and Henrys Law Constant is not appropriate. Based on the physico-chemical properties of ammonium sulfate, water is expected to be the main target compartment. Although ammonium sulfate can be created in the atmosphere from ammonia and sulfur dioxide, this process is limited by atmospheric sulfur dioxide, not by ammonia, which has many natural sources. Particulate ammonium sulfate is removed from air by wet and dry deposition. There is no evidence for photodegradation of ammonium sulfate.

In unsterilized soil, ammonium sulfate is mineralized fairly rapidly, and subsequently nitrified. Nitrification and de-nitrification processes also occur naturally in streams and rivers, as well as in many secondary sewage treatment processes.

Based on the high water solubility and the ionic nature, ammonium sulfate is not expected to adsorb or bioaccumulate to a significant extent. However, mobility in soil may be reduced through ion-ion interactions.

Environmental effects can be assessed in the freshwater and marine environments. In addition, some information is available for soil and sewage treatment micro-organisms, for freshwater sediment, and for the terrestrial environment.

Freshwater Environment

The lowest acute and chronic toxicity values for the three trophic levels are shown in the following table.

Test Type	Trophic Level	Species	Result
Acute	Fish	juvenile *Salmo gairdneri*	LC_{50} (96 h) = 173 mg/l
Acute	Invertebrates	juvenile freshwater snail *Helisoma trivolyis*	LC_{50} (24 h) = 393 mg/l
Acute	Invertebrates	*Daphnia magna*	EC_{50} (96 h) > 100 mg/l
Acute	Aquatic Plants	*Chlorella vulgaris*	EC_{50} (18 d) = 2700 mg/l (cell count)
Chronic	Fish	alevins of *Oncorhynchus gorbuscha*	NOEC (61 d) = 11 mg/l

The PNEC for the freshwater aquatic environment is based upon the lowest observed chronic toxicity result, the NOEC value of 11 mg/l ammonium sulfate for alevins of *Oncorhynchus gorbuscha*. An assessment factor of 100 is appropriate, leading to a freshwater aquatic PNEC of 0.11 mg/l. Supporting information is also available for three juvenile amphibian species. The most sensitive amphibians were 6 week-old *Pseudacris regilla* tadpoles, with a NOEC (10 d) of 82mg/l ammonium sulfate.

Marine Environment

Marine acute data are available for fish, invertebrates and for phytoplankton, the latter being most sensitive. For *Gymnodinium splendens* and *Gonyaulax polyedra , growth* reduction was found at concentrations of 0.7 mg/l and above. No EC50 can be derived. For seawater invertebrates the lowest effect value was obtained for green mussel *Perna viridis* (96h-LC_{50} = 47.7 mg/l). For marine fish the lowest effect value was found for larvae of *Sciaenops ocellatus* with a LC_{50} (10 d) of 27 mg/l.

Micro-organisms in sewage treatment

Nitrification during sewage treatment plant operation involves both sensitive (no growth at 4700 but growth at 94 mg/l ammonium sulfate) and insensitive (growth at 4700 mg/l ammonium sulfate) strains of *Nitrobacter spp*. These results indicate that a NOEC for specific nitrifying bacteria will be greater than 94 mg/l.

In the terrestrial environment, the major effect of repeated ammonium sulfate application is a reduction in soil pH. The most toxic results for specific soil bacteria, for cyanobacteria in rice fields, show less than 50% reduction in nitrogen fixation at 330 kg/ha/yr in the absence of liming. Similar results are seen for plants, with 471 kg/ha/y for 6 years affecting drought resistance in *Picea abies*. The soil fauna is less sensitive, with both *Collembolla* and *Cryptostigmata* numbers increasing under 708 kg /ha/year ammonium sulfate application.

Exposure

According to the statistics of the “International Fertilizer Industries Association” in 2002, approx. 0.76 million tonnes were produced in Germany, approx. 3.95 million tonnes in Western Europe, approx. 3.33 million tonnes in the United States and Canada, and approx. 3.95 million tonnes in Asia including Japan. The world wide production amounts to approx. 17.2 million tonnes per year.

Ammonium sulfate is used primarily as a nitrogen source in commercial fertilizer mixtures or as a direct application fertilizer, which accounts for > 90 % of the total amount. It is further used in a variety of industrial applications and is also approved as a direct food additive in the EU. Non-agricultural products containing ammonium sulfate which are intended for use by the general public (e.g. cleaning products, paints), contain ammonium sulfate levels up to 50 %.

Releases into the environment may occur during production, processing and use. According to measurements in a German chemical plant, releases in air are low. During production and internal processing at one company in the Sponsor country, less than 25 kg were emitted into the air in 2001. No quantitative information on the emission into wastewater or surface water is available for this site; however, any waste material is practically quantitatively recycled at this production site. No data about environmental releases at other production and processing sites are available. Releases into the environment from fertilizer applications may result in some leaching to watercourses from sandy soils, whilst monitoring data from representative European catchments have shown negligible leaching from less acidic, clay and clay loam soils.

Consumer exposure is low.

Ammonium salts and sulfates are abundant in the environment. Ammonium sulfate is a neutralization product of ammonia and sulfuric acid in the atmosphere. Levels of sulfate in air in Canada have been found to range from 3.0 - 12.6 μg/m^3 with a mean of 7.0 μg/m^3. In the USA sulfate concentrations in air ranged from 0.5 - 228 μg/m^3, with the means ranging from 0.8-31.5 μg/m^3. The average daily intake from air would amount to 0.02 - 0.63 μg assuming 20 m^3 of inhaled air per day. In winter indoor sulfate levels (21.6 nmol/m^3) were shown to be similar to outdoor sulfate levels (30.6 nmol/m^3) and were predominantly of outdoor origin. The use of kerosene heaters has been shown to increase indoor sulfate levels (82.7 nmol/m^3); the major form of the indoor sulfate was ammonium sulphate.

Sulfate occurs in drinking water with a median concentration of 24 mg/l and the 99th percentile concentration of 560 mg/l as measured by the U.S. EPA in 2001. Sulfates are natural components of food; ammonium sulfate is "generally recognized as safe (GRAS)" and approved as a food additive in the U.S. and in Europe. From these data it can be seen that consumer exposure to sulfate is low: 453 mg per day via food, 48 mg via drinking water assuming 2 l drinking water per day and 0.63 μg per day via air assuming 20 m^3 respiratory volume. Ammonium sulfate intake via food is 20 mg/day. Endogenous production of ammonia (4000 mg/day) is about 2 orders of magnitude higher than exogenous intake (ammonia and ammonium) via food (20 mg/day), air (<1 mg/day) and water (<1 mg/day).

According to the information provided by the Product Registers, exposure of the general public to ammonium sulfate may occur mainly through the use of fertilizer or horticulture products and to a minor extent through the use of paints and cleaning products.

Exposure of workers to dust during production and storage, loading and unloading of trucks was measured. Eight measurements of respirable dust (including fine dust fraction) were carried out within 8 h (personal air samplers and sampling from the working room), but no analysis for ammonium sulfate performed, assumed as 100%. Results: all measurements showed concentrations close to the detection limit. Personal air samplings for respirable dust showed values < 0.354 - 0.360 mg/m^3 (four samples) and for fine dust < 0.442 - 0.455 mg/m^3 (four samples).

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health: This chemical is currently of low priority for further work because of its low hazard profile.

Environment: The chemical possesses properties indicating a hazard for the environment. These hazards do not warrant further work, as they are related to acute toxicity which may become evident only at high exposure levels. They should nevertheless be noted by chemical safety professionals and users. Although the substance has a low inherent hazard potential for the environment, it degrades in the environment to nitrite. It is recommended that the use of ammonium sulfate as a fertilizer is taken into account when assessing the exposure of nitrite and nitrate to humans through drinking water. The chemical is currently of low priority for further work.

SIDS Initial Assessment Report

1 IDENTITY

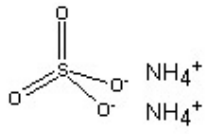
1.1 Identification of the Substance

CAS Number: 7783-20-2

IUPAC Name: Ammonium sulfate

Molecular Formula: $H_8N_2O_4S$

Structural Formula: $(NH_4)_2SO_4$



Molecular Weight: 132.14 g/mol

Synonyms: Ammonium sulphate,
Diammonium sulfate,
Diammonium sulphate,
Mascagnite
Sulfuric acid ammonium salt (1:2),
Sulfuric acid diammonium salt (8CI, 9CI),
Sulfuric acid, diammonium salt,
Sulphuric acid ammonium salt (1:2),
Sulphuric acid diammonium salt (8CI, 9CI),
Sulphuric acid, diammonium salt

1.2 Purity/Impurities/Additives

Today`s commercial ammonium sulfate is generally of high purity (> 99 %), with a water content of ≤ 0.2 % w/w, heavy metals ≤ 5 mg/kg and iron ≤ 5 mg/kg, and free acid ≤ 0.01 % w/w (BASF AG 2002, 2003b).

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Reference
Physical state	White solid	BASF AG, 1999
Melting point	Upon heating in an open system, decomposition begins at temperatures between 150 and 280 °C, depending on experimental conditions and purity of the test substance, and is complete at 336 -357 °C.	Gmelin, 1936; CRC, 2002
Boiling point	decomposes	Gmelin, 1936
Relative density	1.77 at 25 °C	CRC, 2002
Vapour pressure	$4.053*10^{-7}$ Pa (partial pressure of ammonia over solid $(NH_4)_2SO_4$ at 25°C)	Scott and Cattell, 1979
Water solubility	764 g/l at 20 °C	CRC, 2002
Partition coefficient-octanol/water (log Kow)	–5.1 at 25 °C (OECD TG 107) */**	BASF AG, 1988
log Koc	**	
Henry's- Law -constant	not assignable**	
pH	5 - 6	Frank, 1980
pKa-values at 25°C ammonium-ion (base ammonia) sulfuric acid (base HSO_4^-) hydrogensulfate (base sulfate)	 9.21 –3 1.92	Christen, 1973

* The OECD test method 107 applies only to pure, water soluble substances which do not dissociate or associate and which are not surface active. Therefore the validity of this method for ammonium sulfate is uncertain.

** Due to the ionic nature the calculation of log Kow, Henry's-Law-constant, and log Koc via EPISUITE is not appropriate

In aqueous solution, ammonium sulfate is completely dissociated into the ammonium ion (NH_4^+) and the sulfate anion (SO_4^{2-}). Depending on pH, ammonia (NH_3) exists in equilibrium with the ammonium ion (NH_4^+), according to the following relationship:

$$NH_4^+ + H_2O \Leftrightarrow NH_3 + H_3O^+$$

In general, as pH increases, the fraction of the total ammonia which is un-ionized increases. For example, at 5 °C and pH 6.5, 0.0395 % of the total ammonia is present as NH_3. Increasing the pH from 6.5 to 8.5 will increase the un-ionized ammonium by a factor of approximately 100 (Rice and Bailey, 1980). Increasing the temperature will also increase the percentage of unionized ammonium. For example, in seawater at 25 °C and pH of approximately 8.1, approximately 7 % of the total ammonia is present as NH_3 (Holt and Arnold, 1983).

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

According to the statistics of the "International Fertilizer Industries Association" in 2002,
approx. 0.76 mill. tonnes were produced in Germany,
approx. 3.95 mill. tonnes in Western Europe,
approx. 3.33 mill. tonnes in the United States and Canada, and
approx. 3.95 mill. tonnes in Asia including Japan.

The world wide production amounts to approx. 17.2 mill. tonnes per year (IFIA, 2002).

Demand of ammonium sulfate in 2001:
Worldwide: 17.3 mill. tonnes product
Western Europe: 2.8 mill. tonnes product.

Ca. 90 % of ammonium sulfate were used directly as fertilizer or processed as multicomponent mixtures.
Ca. 10 % were used in the chemical industries or processed at metal producing.

In Germany following producers of ammonium sulfate with capacities are known (2001):

BASF AG	ca. 370 KT
Domo Caproleuna GmbH	ca. 250 KT
Different coking plants	ca. 50 KT
EC Erdölchemie GmbH, now BP	ca. 35 KT
Höchst AG, Münchmünster	ca. 4 KT (BASF AG, 2003c)

Ammonium sulfate is coproduct in the production of synthetic-fiber intermediates, such as caprolactam, acrylonitrile, and methyl methacrylate, and in the production of formic acid and acrylamide. The most important source is the production of caprolactam, which is required for nylon6. The conventional caprolactam process produces 2.5 – 4.5 t of ammonium sulfate per tonne of lactam. Of that, 0.3 – 2.8 t is formed in the hydroxylamine – oximation stage and appr. 1.7 t in the Beckmann rearrangement stage. Crystallizers are used in the evaporation of ammonium sulfate solutions that result from caprolactam production (Ullmann, 2000).

Various other uses include applications as (BASF AG, 2002):

- cattle feed
- in cellulose insulation
- in leather tanning
- in herbicide
- in biological treatment plants (as nutrient for bacterial cultures)
- in the chemical industry (for the production of persulfates, and as a nutrient for microorganisms in the production of enzymes and in protein precipitation)
- for the production of fire extinguisher powder and flame proofing agents
- in the production of metals (e.g. chromium), and noble metals (e.g. gold), and as a flotation auxiliary in the treatment of ores
- in the woodworking industry for the production of curing agents for urea-formaldehyde and melamine-formaldehyde resins used in the manufacture of chipboard

- in the leather industry for the production of oozes and deliming agents
- in the paper industry for the production of fire-resistant papers
- in the pharmaceutical industry as a nutrient for microorganisms
- in the textile industry as an additive to dye baths and for flame proofing fabrics, wadding and wicks, and in the production of auxiliaries for textiles processing
- in the wood pulp industry in the production of yeast and sulfite liquor

Further uses reported in the literature include industrial applications such as in water treatment, in shale stabilization and drilling fluids, for galvanizing iron, and for analytical purposes, e.g. for the fractionation of proteins. It is also used in the manufacturing of food additives (aluminum ammonium sulfate) and in the manufacture of viscose silk, ammonia alum, and hydrogen sulfide, wash- and cleaning agents and disinfectants. Combinations of ammonium sulfate and ammonium phosphate or diammonium phosphate are used for fire retardant chemicals (Johnson and Sanders, 1977; Kirk-Othmer, 1984; Budavari, 1996; HSDB, 2002) and for the production of persulfates, flameproofing agents, and fire extinguishing powders; in tanning; in the photographic, textile, and glass industries; and as a nutrient for yeast and bacterial cultures (Ullmann, 2000).

The Danish Product Register (2002) lists 72 products containing 18 tons per annum ammonium sulfate, with the largest category (12 tonnes per annum) being agricultural pesticides generally containing 20 - 50 % ammonium sulfate. Non-agriculture products including products intended for use by the general public (e.g. cleaning products, paints) contain relatively low ammonium sulfate levels (0 - 2 %); the quantities of these products were less than 1 ton per annum ammonium sulfate. The Swiss Product Register (2002) lists 610 ammonium sulfate containing products. The most important use of ammonium sulfate in private households is as fertilizer or in horticulture products, which contain ammonium sulfate concentrations up to 100 %. Cleaning products, paints, laboratory chemicals, and auxiliaries are listed as the most important industrial use categories. The Swedish Product Register (2002) contains 157 products of ammonium sulfate; 26 products thereof are available to consumers. Consumer products are mainly used as fertilizers and fire extinguishing agents.

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Ammonium salts and sulfates are abundant in the environment. Ammonium sulfate is a neutralization product of ammonia and sulfuric acid (WHO, 1986). Sulfate results from the oxidation of elemental sulfur, sulfide minerals, and organic sulfur, e.g. through the combustion of sulfur-containing fuels. Sulfates are found almost universally in natural waters at concentrations ranging from a few tenths to several thousand mg/l (EPA, 2002).

In the frame of the German water quality monitoring program ammonium nitrogen concentrations were measured. In 2000, the rivers Danube, Oder, Weser, Rhine, and Elbe showed concentrations (50^{th} percentile) ranging from 0.04 to 0.07 mg/l (UBA, 2003).

UBA (2003) states that in Germany 624 000 t ammonia were emitted to air in 1999. Livestock farming (ca. 83 %) and fertilizer use (ca. 12 %) are the main sources for the emissions. Industrial releases of ammonia were less than 3 %. In the atmosphere, ammonia can react with sulfur dioxide to produce ammonium sulfate contained in atmospheric aerosols. These can return to the earth`s surface as wet or dry deposition (Scott and Cattell, 1979; Gmur, Evans and Cunningham, 1983). For

example, long term averaged monthly ammonium and sulfate measured in rainwater from a rural coastal island located 8 - 10 km from the NL mainland were 2.68 mg/l and 6.65 mg/l respectively, with seasonal variations showing higher values in late winter/early spring, and lower values in the autumn. Extensive cattle raising on the island was used to explain these values being higher than those found at a corresponding rural site 4 km southeast of Amsterdam (Weijers and Vugts, 1990).

In 1998, about 437 000 t ammonia were deposited in Germany (UBA 2003).

Ammonium and sulfate concentrations in outdoor air have been determined from particulate sampling. For example, sampling equipment located on the roofs of three workplaces in the northeastern USA found 84 hour average collection results ranging from 1573 - 1840 ng/m^3 for ammonium and 3800 - 5214 ng/m^3 for sulfate, in a 9 month period from June 1988 (Sinclair et al., 1992). A representative regional air quality site in VA, USA, had mean summertime ammonium concentrations of 2243 ng/m^3 (range 551 - 5274 ng/m^3), while samplers placed outside of houses had an average ammonium concentration of 2329 ng/m^3 (range 0 - 6101 ng/m^3) The results are based on twenty-four hour particle samples collected during the period from 15 May through 15 September for 1995 and 1996 (Leaderer et al., 1999).

Releases into the environment may occur during production, processing and use.

Releases from manufacturing and processing:

According to measurements in a German chemical plant, releases in air are low.

During production and internal processing at BASF AG, Ludwigshafen (Germany), less than 25 kg were emitted into the air in 2001. No quantitative information on the emission into wastewater or surface water is available for this site; however, any waste material is practically quantitatively recycled at the Ludwigshafen production site (BASF AG, 2003a).

Releases to the environment could potentially occur from sites where e.g. pesticides, cleaning products, paints, fire extinguishers etc. are manufactured. However, no data relating to such other production and processing sites and of emissions are available.

Releases from use of ammonium sulfate containing products:

Its main use, in fertilisers applied to land, may result in some leaching to streams.

Approximately 90 % of ammonium sulfate is used in products applied directly to land, for example as fertilisers or as a component in herbicides. Although, especially in sandy soils at low pH, this may result in some leaching to watercourses, detailed monitoring in forest soils in six representative European catchments has shown negligible leaching from less acidic, clay and clay loam soils (Sveda, Rechcigl and Nkedi-Kizza, 1992; Berden and Nilsson, 1996; Perrin et al., 1998). Leaching has been shown to be dependent upon the application regime as well as on the soil type (Carnol, Ineson and Dickinson, 1997; Carnol et al., 1997). In a study in the Bear Brook catchment in Maine, USA, ^{15}N was used to demonstrate that less than 1 % of the nitrogen found in stream water originated from fertiliser use (Nadelhoffer et al., 1999). In this catchment a fourfold increase in N inputs to 2400 mol N/ha/year caused no increase in DOC in the stream (David, Vance and Kahl 1999).

Ammonium sulfate will not volatilise from soil in significant amounts.

The intensity of NH_3 volatilisation from ammonium sulfate is depending on fertilizer management practice, soil characteristics and weather conditions. Shahandeh, Cabrera and Summer (1992), Moal et al. (1994) and Debreczeni and Berecz (1998), describe the release of ammonia (NH_3) up to 60 % resulting from the breakdown of ammonium sulfate added as fertilizer. According to the European Environmental Agency (2004) the NH_3 loss coefficient for ammonium sulfate is on average of 0.02 (acid soils) and 0.20 (calcareous soils) NH_3-N per kg fertilizer N.

Other uses of ammonium sulfate (*cf* section 2.1) may result in an exposure of surface waters. However, no release data are available.

2.2.2 Photodegradation

There is no evidence for photodegradation of ammonium sulfate. However, ammonium sulfate may be formed in atmospheric aerosols (occurrence predominantly in the fraction < 2.5 µm) from reaction between ammonia and sulfuric acid (from SO_2 emissions). SO_2 concentrations control the ammonium sulfate formation (Scott and Cattell, 1979; Gmur, Evans and Cunningham, 1983).

2.2.3 Stability in Water

In aqueous solution, ammonium sulfate is completely dissociated into the ammonium ion (NH_4^+) and the sulfate anion (SO_4^{2-}). Hydrolysis of ammonium sulfate does not occur.

2.2.4 Transport between Environmental Compartments

Due to the salt-character of the substance the calculation of a fugacity model is not appropriate. Based on the physico-chemical properties of ammonium sulfate, water is expected to be the main target compartment.

Because of the chemical structure of ammonium sulfate, the Henrys Law Constant is not assignable.

Based on the high water solubility a low geoaccumulation potential and high mobility in soil is to be expected. However, due to ion-ion interactions it is to be expected that mobility in soil is significantly reduced.

Ammonium sulfate will not volatilise from soil.

2.2.5 Biodegradation

In unsterilized soil, ammonium sulfate is mineralized fairly rapidly, and subsequently nitrified. Nitrification and de-nitrification processes also occur naturally in streams and rivers, as well as in many secondary sewage treatment processes (WHO, 1986).

2.2.6 Bioaccumulation

Based on a log K_{ow} of –5.1 (measured; BASF AG, 1988), bioaccumulation is not expected.

2.2.7 Other Information on Environmental Fate

2.2.7.1 Stability in soil

Effects covered in Section 2.2.3 will also occur in soil porewater. Ammonia from ammonium sulfate decomposition can be released from soils, especially if applied fertiliser is not covered by soil. For the amounts of ammonia volatilisation see section 2.2.1. Ammonium remaining in soil is largely adsorbed onto positively charged clay particles, and will undergo nitrification and denitrification as part of the nitrogen cycle and be taken up by plants via nitrogen fixation (WHO, 1986). Sulfate can also be retained in soil, both by incorporation into organic matter (e.g. as sulfate esters of humic acids) and adsorbed to soil particles such as hydrous iron and aluminum sesquioxides (EPA, 2002).

2.3 Human Exposure

2.3.1 Occupational Exposure

Exposure of workers to ammonium sulfate may occur during production, transport and processing, or through the professional use of ammonium sulfate containing products. Main exposure routes are the respiratory route (inhalation of aqueous aerosols or dust), or dermal contact with the solid.

Exposure of workers to dust during production and storage, loading and unloading of trucks was measured. Eight measurements of respirable dust (including fine dust fraction) were carried out within 8 h (personal air samplers and sampling from the working room), but no analysis for ammonium sulfate performed, assumed as 100 %.

Results: all measurements showed concentrations close to the detection limit. Personal air samplings for respirable dust showed values < 0.354 - 0.360 mg/m^3 (four samples) and for fine dust < 0.442 - 0.455 mg/m^3 (four samples), (BASF, 2004, workplace measurements)

These numbers show a generally low exposure toward ammonium sulfate during production and filling.

Exposure of workers during production is controlled (cf. also the above paragraph). Generally, the production units are inspected and repaired annually but also in shorter intervals if necessary. When the production site is opened e.g. for repair or cleaning, appropriate protective measures are applied. Work on the opened system is done only by authorized staff wearing breathing protection (particle filters), chemical resistant protective gloves and safety glasses with side-shields. Only well trained workers are involved in maintenance of the system. Regular instructions will ensure work safety. These safety instructions e.g. for cleaning of the reactor, are documented and kept centrally in the control room of the production site.

2.3.2 Consumer Exposure

Ammonium salts and sulfates are abundant in the environment (see also above general exposure section). Ammonium sulfate is a neutralization product of ammonia and sulfuric acid in the atmosphere (WHO, 1986). Levels of sulfate in air in Canada have been found to range from 3.0 - 12.6 µg/m^3 with a mean of 7.0 µg/m^3. In the USA sulfate concentrations in air ranged from 0.5 -

228 µg/m^3, with the means ranging from 0.8 - 31.5 µg/m^3. The average daily intake from air would amount to 0.02 - 0.63 µg assuming 20 m^3 of inhaled air per day (WHO, 1996). In winter indoor sulfate levels (21.6 nmol/m^3) were shown to be similar to outdoor sulfate levels (30.6 nmol/m^3) and were predominantly of outdoor origin (*cf* section 2.2.1; Sinclair et al., 1992). The use of kerosene heaters has been shown to increase indoor sulfate levels (82.7 nmol/m^3); the major form of the indoor sulfate was ammonium sulfate (Leaderer et al., 1999).

Sulfate occurs in drinking water with a median concentration of 24 mg/l and the 99th percentile concentration of 560 mg/l as measured by the U.S. EPA in 2001. The average daily intake of sulfate in food in the US has been estimated to be 453 mg (EPA 2002). From this data it can be seen that consumer exposure to sulfate is low: 453 mg per day via food, 48 mg per day via drinking water assuming 2 l drinking water per day and 0.63 ug per day via air assuming 20 m^3 respiratory volume.

Sulfates are natural components of food; ammonium sulfate is “generally recognized as safe (GRAS)” and approved as a food additive in the U.S. (FDA 2003) and in Europe (E 517; EU, 1995).

Ammonium (ammonia and ammonium) intake via food is 18 mg/day, via air and water < 1 mg/day.Endogenous production of ammonia (4000 mg/day) is about 2 orders of magnitude higher than exogenous intake.(WHO, 1986, 1996) According to the information provided by the Product Registers, exposure of the general public to ammonium sulfate may occur mainly through the use of fertilizer or horticulture products and to a minor extent through the use of paints and cleaning products (*cf* Chapter 2.1).

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

The following endpoint studies with ammonium sulfate were not available,

- fertility
- developmental toxicity
- genotoxicity in vivo

As ammonium sulfate dissociates in biological systems studies with other ammonium and sulfate salts can be used to cover these endpoints: A screening study according to OECD TG 422 with ammonium phosphate as analogue substance, which forms ammonium ions in aqueous solutions is available. Fully valid fertility studies with analogue compounds containing sulfate ions are however lacking. Two limited studies with sodium sulfate can be used for assessment of fertility and developmental toxicity, however, in none of these studies have the fetuses been examined histologically.

There are no in vivo data on genotoxicity for ammonium sulfate. To bridge the data gap, data for ammonium chloride, which dissociates in aqueous media to form ammonium ions, as does ammonium sulfate, will be used. However, data on sulfate ions are not available.

3.1.1 Toxicokinetics, Metabolism and Distribution

In aqueous environments, such as the body the ammonium sulfate is completely dissociated into the ammonium (NH_4^+) and the sulfate (SO_4^{2-}) ions. At physiological pH in aqueous media, the ammonium ion is in equilibrium with un-ionized ammonia, according to the following equation:

$$NH_4^+ + H_2O \Leftrightarrow NH_3 + H_3O^+$$

The ammonium ion serves a major role in the maintenance of the acid-base balance. In the normal pH range of blood, the NH_4^+ / NH_3 is about 100 (WHO, 1986).

An ammonium ion via the equilibrium with ammonia is readily taken up. Some evidence exists also for an active transport of the ammonium ion from the intestinal tract. It was shown that ammonia transport by the human colon still occurred when the luminal pH was reduced to 5, where non-ionized ammonia would be virtually absent (WHO, 1986).

Absorbed ammonium is transported to the liver and metabolized to urea and excreted via the kidneys. Minor amounts of nitrogen are incorporated in the physiological N-pool (WHO, 1986).

Absorption of sulfate depends on the amount ingested. 30 - 44 % of sulfate was excreted in the 24-h urine after oral administration of magnesium or sodium sulfate (5.4 g sulfate) in volunteers. At high sulfate doses that exceed intestinal absorption, sulfate is excreted in feces. Intestinal sulfate may bind water into the lumen and cause diarrhoea in high doses. Sulfate is a normal constituent of human blood and does not accumulate in tissues. Sulfate levels are regulated by the kidney through a reabsorption mechanism. Sulfate is usually eliminated by renal excretion. It has also an important role in the detoxification of various endogenous and exogenous compounds, as it may combine with these to form soluble sulfate esters that are excreted in the urine (EPA, 2002).

Studies in Animals

In vivo Studies

In rabbit, hamster and guinea pig studies it was demonstrated that S^{35}-labelled ammonium sulfate aerosols with a size of 0.3 and 0.6 µm (MMAD) reached the lung, however a substantial proportion of the compound was found in the nose. The clearance from the lung (via the blood and urinary tract) was determined to be 18 to 20 minutes. From the collectable sulfate in the urinary tract 95 % was excreted within 6 hours. The results of clearance studies suggested that there was no species difference. The induction of aryl hydrocarbon hydroxylase (an enzyme that acts in the metabolism of benzo(a)pyrene and other carcinogens) in the lung is not inhibited by ammonium sulfate (there are reports of other air pollutants to cause this effect) (EPA, 1978; Godleski et al., 1984).

Studies in Humans

In vivo Studies

There are no in vivo studies in humans available with ammonium sulfate.

In vitro Studies

There are no in vitro studies with human tissue available with ammonium sulfate.

Conclusion

In aqueous media, ammonium sulfate dissociates in the ammonium and sulfate ions (NH_4^+, SO_4^{2-}). These can be taken up into the body by the oral and respiratory routes. Absorbed ammonium is transported to the liver and there metabolized to urea and excreted via the kidneys. Ammonium is

also an endogenous substance that serves a major role in the maintenance of the acid-base balance. Minor amounts of ammonium nitrogen are incorporated in the physiological N-pool. Sulfate is a normal intermediate in the metabolism of endogenous sulfur compounds, and is excreted unchanged or in conjugated form in urine.

3.1.2 Acute Toxicity

Studies in Animals

Inhalation

The acute inhalation toxicity of ammonium sulfate aerosols (average diameter 1 - 3 µm) is very low with 8-h LC_{50} values of greater than 900 mg/m^3 for guinea pigs. Rats were exposed repeatedly for 8 h/d to 1000 - 1200 mg/m^3 (average diameter 2 - 3 µm) without mortality. No specific signs of toxicity were reported from these studies (Pepelko, Mattox and Cohen, 1980).

Mucociliary clearance was neither significantly affected in male rabbits that were exposed to 2 mg/m^3 for one hour (mass median diameter: 0.4 µm) (Schlesinger, 1984), nor in sheep that were exposed to 1.1 mg/m^3 (< 1 µm) for 20 minutes (Sackner et al., 1981) nor in rats exposed to 3.6 mg/m^3 (0.4 µm) for 4 h (Phalen et al., 1980).

Pulmonary resistance was slightly increased and compliance was statistically significantly decreased in guinea pigs exposed to 0.5 - 9.5 mg/m^3 for one hour (Amdur et al., 1978). Pulmonary mechanics were not altered in dogs breathing ammonium sulfate aerosol at a concentration of 4.1 mg/m^3 for 4 h (Sackner et al., 1981).

Dermal

LD_{50} values of > 2000 mg/kg bw are reported for rats and mice after dermal application of ammonium sulfate. Details on clinical signs and necropsy findings were not given (Yamanaka et al., 1990).

Oral

In rats, the oral LD_{50} was determined to be 4250 mg/kg bw (95 % confidence limits: 3788 - 4769). At doses near to or exceeding the LD_{50} value, staggering, prostration, apathy, and laboured and irregular breathing were observed immediately after dosing. On the next day, secretion out of eyes and mouth, and reddened eyes and nose were seen. In the post-exposure observation days the surviving animals were without clinical symptoms. No clinical signs were noted at doses up to and including 2500 mg/kg bw (BASF AG, 1969).

In another study similar to OECD TG 423, LD_{50} values of about 2000 mg/kg bw are reported for rats and of > 2000 mg/kg bw in mice (Yamanaka et al., 1990). In a full LD_{50} test according to the Toxicity Guidelines of Japan (1984) an oral LD_{50} for mice of 3040 mg/kg bw was obtained. Details on clinical signs and necropsy findings were not given (Yamanaka et al., 1990).

Studies in Humans

Inhalation

As ammonium sulfate forms in the environment from ammonia and sulfur dioxide (see chapter "environmental exposure"), in the 1970s and 1980s several studies of acidic aerosols on the lung function in humans have been conducted. Most of the studies were performed with low, environmentally relevant concentrations up to about 1 mg ammonium sulfate/m^3.

Exposure of 13 healthy male volunteers to ammonium sulfate aerosol for four hours at a concentration of 0.133 mg/m^3 (MMAD 0.55 µm) produced no significant effects related to 19 measured pulmonary parameters, including specific airway resistance, forced vital capacity, and forced expiratory flow (Stacy et al., 1983).

No significant changes in pulmonary parameters were reported from healthy and asthmatic volunteers exposed for 2 hours to ammonium sulfate (0.1 - 0.3 mg/m^3; MMAD 0.3 - 0.6 µm) (Avol et al., 1979).

At 1 mg sulfate/m^3 (MMAD 1 µm) ammonium sulfate inhaled for 16 minutes produced a small but significant decrease in expiratory flow in healthy subjects. Carbachol induced bronchoconstriction was slightly enhanced (no statistical evaluation) (Utell, Morrow and Hyde, 1982). A similar study with asthmatics exposed to 1 mg sulfate/m^3 as ammonium sulfate for 16 minutes found neither a significant decrease in specific airway conductance nor in forced expiratory volume in one second. Flow rates were not altered (Utell et al., 1983).

Pulmonary function (measured by body plethysmography and spirometry), and bronchial reactivity to metacholine were not affected in 20 non-smoking volunteers after a 4-hour exposure to 0.5 mg/m^3 ammonium sulfate. The exposures included two 15-minute light to moderate exercise stints per day in the exposure chamber (Kulle et al., 1984).

In senior asthmatics a 40 min exposure to 70 µg ammonium sulfate/m^3 (MMAD 0.6 µm) did not have any significant effect on pulmonary function (Koenig et al., 1993).

At 1 mg ammonium sulfate/m^3 a decrease in pulmonary flow resistance and decreased dynamic lung compliance were found in 4 healthy volunteers after 120 min of exposure (Frank et al., 1977).

Oral

A case of a fatal poisoning with ammonium sulfate was reported from an 85-year-old woman after drinking an unspecified amount of ammonium sulfate dissolved in beer in a suicidal attempt. Heart, lung, liver and kidney did not show any pathological findings on macro- and microscopical examination. There was mild petechial hemorrhage in the gastric fundic mucosa without any erosion or corrosion. In serum, ammonium and sulfate ions were significantly increased (25 000 µg/dl and 12.35 mEq/l, resp.; normal range 30 - 80µg/dl and 0.25 - 0.35 mEq/l, resp.) (Sato, Gonmori and Yoshioka, 1999).

Because of their osmotic activity sulfate salts draw water into the lumen of the bowel and produce diarrhea in high doses (EPA, 2002).

Conclusion

Ammonium sulfate is of relatively low acute toxicity (LD_{50}, oral, rat: 2000 - 4250 mg/kg bw; LD_{50} dermal, rat/mouse > 2000 mg/kg bw; 8-h LC_{50}, inhalation, rat > 1000 mg/m^3). Clinical signs after oral exposure included staggering, prostration, apathy, and laboured and irregular breathing immediately after dosing at doses near to or exceeding the LD_{50} value. In humans, inhalation exposure to 0.1 – 0.5 mg ammonium sulfate/m^3 aerosol for two to four hours produced no pulmonary effects. At 1 mg ammonium sulfate/m^3 very slight pulmonary effects in the form of a decrease in expiratory flow, in pulmonary flow resistance and dynamic lung compliance were found in healthy volunteers after acute exposure.

3.1.3 Irritation

Skin Irritation

Studies in Animals

In rabbits (number not reported), single semiocclusive exposure for 20 hours or multiple exposures (8 h/day) on five consecutive days to the neat test substance (purity not reported) did not elicit any adverse effects on intact skin. Single exposure for 8 hours to scarified skin caused only slight redness, and slight edema. These effects were completely reversible within 8 days (BASF AG, 1968).

In two rabbits, single exposure of up to 20 hours to an 80 % aqueous preparation of ammonium sulfate ("chemically pure") was not irritating to the intact skin (BASF AG, 1969).

Studies in Humans

There are no studies in humans available.

Eye Irritation

Studies in Animals

In two rabbits, slight edema and conjunctival redness was noted one hour after instillation of 50 mm^3 of the neat test substance ("chemically pure") into the conjunctival sac. No edema, but still slight redness was present at 24 hours. In the eyes treated with talcum, effects similar to the treatment with the test substance were found one and 24 hours after exposure. No effects were noted at day 8 (BASF AG, 1969).

Studies in Humans

There are no studies in humans available.

Respiratory Tract Irritation

Studies in Animals

There are no animal studies available.

Studies in Humans

see 3.1.2

Conclusion

Neat ammonium sulfate was not irritating to the skin and eyes of rabbits.

3.1.4 Sensitisation

There is no data available.

3.1.5 Repeated Dose Toxicity

Studies in Animals

Inhalation

The repeated dose toxicity after inhalation of ammonium sulfate aerosols was studied in rats, guinea pigs, and hamsters, using non-standard protocols. In most of the studies the animals were pretreated with intratracheal instillations, only one concentration was tested, the test protocol was not validated, the scope of the examinations was limited or the animals were examined not immediately after the last exposure, so only one study is considered to be sufficiently reliable.

In a 14-day inhalation study, 10 male rats were exposed to an ammonium sulfate aerosol for 8 hours/day at a concentration of 300 mg/m^3 (count mean particle diameter: 1 - 2 µm). The concentration used was determined on the basis of a preliminary study with rats, which were exposed for 3 consecutive days to the maximum attainable concentration of ammonium sulfate (1000 - 1200 mg/m^3; 8 hours/day; count mean particle diameter: 2 - 3 µm). No adverse effects were noted in this pre-test. In the main study, arterial blood gases were measured after 1, 3, 7, and 14 days. Pulmonary function tests were performed after 14 days of exposure and histological examinations were performed at the end of the exposure period. The exposure did not result in any significant changes in lung morphology, lung volumes, and arterial blood gases. The histological examinations did not reveal any changes in the trachea, bronchial lymph nodes, and lungs and the exposure had no adverse effects on body weight. A NOEL of 300 mg/m^3 for toxicity to the lower respiratory tract can be deduced from the study (Pepelko, Mattox and Cohen, 1980).

In an inhalation study consisting of two separate experiments with 160 male hamsters, in the ammonium sulfate group and control group, resp, the incidence or severity of pneumonitis or pulmonary fibrosis was not significantly increased after 15 weeks of exposure (6 hours/day, 5 days/week) to approximately 0.2 mg ammonium sulfate/m^3. The animals were examined after 2 years. Also, no significant pathological changes of organs were observed. This inhalation did increase the incidence of emphysema as examined microscopically but not the severity. The increase in emphysema incidence was not statistically significant (9.0 vs 15.2 %; EPA, 1978). Another publication of this study described an increase in emphysema incidence, which is however of doubtful statistical significance. It was based on only 80 animals per group (8.6 vs 16.1 %; Godleski et al., 1984). Due to the higher number of animals used, the EPA results are more reliable and 0.2 mg ammonium sulfate/m^3 cannot be regarded as a LOAEL. However it seems not acceptable to take 0.2 mg/m^3 as the NOAEL from this study because the animals were not examined immediately after exposure but only after 2 years.

Studies in animals pre-treated with intratracheal instillations:

Loscutoff et al. (1985) exposed 27 - 29 male Sprague-Dawley rats/group for 5 or 20 days (6 hours/day, 5 days/week) to approximately 1 mg/m³ of ammonium sulfate aerosol (MMAD: 0.42 µm) after receiving an intratracheal instillation of saline. The exposure resulted in an increased residual volume and functional residual capacity indicative of a mild pulmonary emphysema.

In a further study, 20 Sprague-Dawley rats were exposed for 20 days (6 hours/day, 5 days/week) to approximately 1.03 mg/m³ of ammonium sulfate aerosol (MMAD: 0.42 µm) after receiving an intratracheal instillation of saline or elastase. The exposure resulted in a measurable degree of enlargement of alveoli, alveolar ducts and sacs (Busch et al., 1984). These changes may be indicative of beginning lung emphysema; clear changes were, however, only found in elastase pretreated animals.

A similar study was carried out with 15 Hartley guinea pigs, exposed for 20 days (6 hours/day, 5 days/week) to approximately 1.03 mg/m³ of an ammonium sulfate aerosol (MMAD: 0.42 µm) after receiving an intratracheal instillation of saline. The exposure resulted in an apparent alteration in the secretory activity characterized by hypertrophy and hyperplasia of non-ciliated epithelial cells, with an increased number of secretory granules per cell. Changes comparable to those in the rat study were not seen (Busch et al., 1984). The reported changes may be seen as signs of an inflammatory response.

In another study, the examination of the lung function of 29 Hartley guinea pigs exposed for 5 or 20 days (6 hours/day, 5 days/week) to approximately 1 mg/m³ of ammonium sulfate aerosol (MMAD: 0.42 µm) after receiving an intratracheal instillation of saline revealed no adverse effect (Loscutoff et al., 1985).

In a further study by the same working group (Smith et al., 1989), groups of rats were pre-treated intratracheally with either physiologic saline or porcine pancreatic elastase. Animals of each pre-treatment group were exposed to filtered air (controls), or to 0.5 mg/m^3 ammonium sulfate for 5 hours/day and 5 days/week for 4 months (n = 15), 8 months (n = 15), or for 8 months plus 3 months recovery (n = 14), There were no signifycant differences in final body weights or in the investigated immunologic parameters measured after 4 months (spleen weight, mitogenic response of spleen cells, peripheral blood lymphocytes, distribution of T cells in the spleen). No differences were found in vital capacity, total lung capacity, time constant, CO_2-diffusion capacity, residual volume, functional residual capacity, or N_2-slope measured after 4 months. The quasistatic compliance was slightly, though significantly reduced.

Main results from the saline-pretreated groups are summarized in the following table (results from elastase-pretreated animals are not included):

Table 2: Morphological lung effects in saline-pretreated rats after exposure to ammonium sulfate for 4 months, 8 months, or 8 months plus 3 months recovery:

	4 months treatment		**8 months treatment**		**8 months treatment plus 3 months recovery**	
number of animals with effect / total number of animals	Air	$(NH_4)_2SO4$	Air	$(NH_4)_2SO_4$	Air	$(NH_4)_2SO_4$
Emphysema	2/15	0/15	0/13	2/15	2/13	4/14
Alveolar birefringence	8/15	0/15	9/13	15/15*	10/13	12/14
Bronchiolar epithelial hyperplasia	6/15	13/15*	0/13	0/15	0/13	0/14
NEC[a] counts (cells/standard area)	27.7	32.7*	24.8	30.0*	26.4	25.9
Chord length						
Mean (mm)	4.66	5.07*	4.28	4.45	3.87	4.14*
Median (mm)	4.29	4.47*	3.71	3.99	3.60	3.92*

[a]Nonciliated epithelial cells

* $p < 0.05$

After 4 months, 2 control animal and none of the ammonium sulfate treated animals showed emphysema. After 8 months, the incidence of emphysema in the treated group (2 out of 15 animals) was equal to that in the control group after 4 months, indicating that the incidence of emphysema was similar in the treated and control animals.

An increase in the incidence of emphysema in the treated group after 8 months plus 3 months recovery (4 out of 14 animals) versus the control group (2 out of 13 animals) is, with regard to the small group size, difficult to interpret, as there were no indications for such an effect from the 4- and 8 months data.

The same problem is apparent with the data for alveolar birefringence. The value of 53 % (8 out of 15 animals) in the control group after 4 months appears to be extraordinarily high, as physiological saline should have had no effects in the lungs, and also, because the animals were still quite young. In contrast, the ammonium sulfate-treated animals had a 0 % incidence of alveolar birefringence at the same time point. After 8 months, all of the ammonium-sulfate treated animals showed alveolar birefringence (a phenomenon that is indicative of fibrosis according to the study authors), as compared with 67 % in the control group. It is surprising that during recovery more control animals showed this effect, whilst the number of animals with this effect dropped in the ammonium-sulfate treated group. Overall, the data appear to be very inconsistent. Moreover, it is questionable whether “alveolar birefringence” is synonymous with “interstitial fibrosis”.

At 4 months, the incidence of bronchiolar hyperplasia in the controls is high (40 %), but it is even higher in the ammonium sulfate-treated rats (87 %). Surprisingly, no hyperplasia (neither in controls nor in treated rats) was observed after 8 months, although exposure to the test substance continued. It remains open as to what exactly was measured, as “all the cell types present in that area” and the “depth of the epithelium” were included in the measurements. A scientific evaluation of the reported findings is therefore impossible.

The mean and median chord lengths were statistically significantly increased after 4 months and after recovery but only numerically after 8 months, also indicating a great variation in these finding. Nonciliated epithelial cell counts were increased after 4 and 8 months but not after recovery. In conclusion, it remains open whether the results discussed above are related to a bad health status of the animals, or to rather unspecific or inappropriate investigation methods. Nevertheless, it is evident that the findings cannot clearly be attributed to the test substance and that they are of limited relevance for the toxicological assessment of ammonium sulfate.

Exposure of rabbits for 14 days, 2 h/d to 2 mg ammonium sulfate/m^3 did not result in a change in respiratory region clearance (Schlesinger, 1989).

Dermal

There were no dermal studies available.

Oral

Fischer 344 rats (10 rats/sex/dose) were exposed during 13 weeks to diets containing 0, 0.38, 0.75, 1.5 or 3 % ammonium sulfate (corresponding to 0, 222, 441, 886, 1792 mg/kg bw/day in males and to 0, 239, 484, 961, 1975 mg/kg bw/day in females) No substance-related changes between the treatment groups and controls were found in body weights, haematology and serum parameters, or in the histological examinations (brain, heart, lung, liver, kidney, adrenal gland, spleen, testes, thymus) Relative and absolute kidney weights were increased in both male and female animals in the highest dose group. The authors did not judge this as adverse, because there were no histopathologic effects seen in the kidneys. The relative testes weight was significantly increased at all doses, but no histological effects were found. Male animals of the highest dose group exhibited

diarrhoea during the administration period. According to the authors the NOAEL (male) was 886 mg/kg bw/day and the NOAEL (female) was 1975 mg/kg bw/day (Takagi et al., 1999). The main limitation of the study is the low number of organs examined histopathologically compared to a standard 13-week guideline study.

Studies in Humans

There were no studies in humans available.

Conclusion

A 14-day inhalation study on rats exposed to 300 mg/m^3, the only tested dose, did not report histopathological changes in the lower respiratory tract. As the respiratory tract is the target organ for inhalation exposure, the NOEL for toxicity to the lower respiratory tract is 300 mg/m^3.

The NOAEL after feeding diets containing ammonium sulfate for 13 weeks to rats was 886 mg/kg bw/day. The only toxicity sign found was diarrhea in male animals of the high-dose group (LOAEL: 1792 mg/kg bw/day).

3.1.6 Mutagenicity

Studies in Animals

In vitro Studies

Ammonium sulfate was not mutagenic in the standard plate and pre-incubation Ames test performed in 4 strains of *Salmonella typhimurium* (TA1535, TA100, TA1537, TA98) with and without a metabolic activation system up to and including the maximum tested concentration of 5000 µg/plate. No cytotoxic effects were observed (BASF AG, 1989b). Ammonium sulfate was also not mutagenic in *Salmonella typhimurium* strains TA1535, TA1537 and TA1538, and in *Saccharomyces cerevisiae* D4 with and without metabolic activation systems. Again, no cytotoxic effects were observed up to the highest tested concentration of 50 000 ppm (Litton Bionetics, 1975).

Treatment of Chinese Hamster Ovary (CHO) (Tuschy and Obe, 1988) cells and treatment of human lymphocytes (Obe, Jonas and Schmidt, 1986) with 3.2 M (423 mg/ml) ammonium sulfate in the absence of a metabolic activation system, did not result in chromosomal aberrations. However, ammonium sulfate enhanced the frequency of chromosome type aberrations, which had been induced by the restriction endonuclease Alu 1. A similar effect was observed with other salts (magnesium chloride, calcium chloride and sodium chloride), and is not indicative of a mutagenic effect of ammonium sulfate (Obe, Jonas and Schmidt, 1986; Tuschy and Obe, 1988).

In vivo Studies

The dossier of ammonium chloride reported negative results in a micronucleus test in mice (SIAM 16, 2003). This micronucleus test was conducted with bone marrow in ddY mice (Hayashi et al., 1988). Animals had received a single and 4 times i.p. injection. A single dose test and 4 times dose test were dosed with 62.5 - 500 mg/kg and 31.3 - 250 mg/kg as MTD (maximum tolerated dose), respectively, with mitomycin C as positive control. No increase of erythrocytes with micronuclei was observed at any group.

The potential of ammonium sulfate to induce mutagenic effects *in vivo* is considered to be negligible, because there is no evidence of a mutagenic effect from *in vitro* studies. There are no in vivo studies available with ammonium sulfate.

Studies in Humans

There is no data available.

Conclusion

Ammonium sulfate was not mutagenic in bacteria (Ames test) and yeasts with and without metabolic activation systems. It did not induce chromosomal aberrations in mammalian or human cell cultures. No in vivo genotoxicity tests are available. Based on the negative results from in vitro studies and the negative results in the micronucleus test in vivo with ammonium chloride a mutagenic activity of ammonium sulfate in vivo is unlikely.

3.1.7 Carcinogenicity

In vitro Studies

There is no data available.

In vivo Studies in Animals

Inhalation

There is no data available.

Dermal

There is no data available.

Oral

The effect of various salts including ammonium sulfate on ornithine decarboxylase (ODC) as a marker for tumor promotion in rat stomach mucosa was reported by Furihata et al. (1989). A 73 fold increase in enzyme activity was measured with a maximum at 16 hours after a single oral gavage (500 - 2660 mg/kg bw). In comparison, an equimolar dose of NaCl (25.7 mmol = 1500 mg/kg bw) induced a 248 fold increase in enzyme activity. The authors concluded that the various tested salts may have the capability of tumor promotion in the glandular stomach of rats. In this study, very high salt concentrations were given in a bolus directly into the stomach. High salt concentrations can denature proteins resulting in cell injury or cell death with subsequent cell proliferation as a repair mechanisms causing the increase in ODC activity which is a normal, secondary physiological response to cellular injury and death. Therefore, the study did not provide supporting evidence for carcinogenicity of ammonium sulfate.

Studies in Humans

Respiratory Tract

In a nested case-control study within the active workforce of a large chemical manufacturing firm over a 23-year period, no association with Hodgkin`s disease was identified for 11 chemicals (including ammonium sulfate) (Swaen et al., 1996).

Conclusion

Similarly to other salts, high doses of ammonium sulfate may have the capability of tumor promotion in the rat stomach; it is, however, much less potent than sodium chloride when tested under identical conditions.

3.1.8 Toxicity for Reproduction

There are no valid studies available in which ammonium sulfate has been tested for its effects on fertility and development. The following are available for the ammonium and the sulfate ion.

Effects on Fertility

In a gavage study performed according to OECD TG 422 with diammonium phosphate (250, 750, 1500 mg/kg) in CD rats (10 females and 5 males/group), no mortality was observed up to the highest tested dose level of 1500 mg/kg bw/day. Some treatment-related effects on hematology parameters (reduction in activated partial thromboplastin time for males at 750 or 1500 mg/kg/day were evident, and bodyweight gain was temporarily reduced in the high dose group (in males week 0 - 5, in females week 1). No treatment-related signs of clinical toxicity were observed. Mating performance and fertility were unaffected by parental exposure to diammonium phosphate (The Fertilizer Institute, 2002).

Groups of 10 female ICR mice were given sodium sulfate in drinking water at levels of 0, sodium control, 625, 1250, 2500 or 5000 mg sulfate/l (ca. 250 - 850, 480 - 2040, 1270 - 4320, 1790 - 6560 mg sulfate/kg bw) beginning one week prior to breeding and up to 14 days during lactation. At day 21 p.p. the pups were weaned and the dams were rebred at first estrus immediately following weaning. Only animals that whelped during each parity were used in the analysis. The effective number of dams per group was low: 4 - 9 in the first parity, and 4 in the second parity. Control mice, receiving only distilled water, consumed significantly less water than mice receiving sulfate treatments, and sodium-control mice drank significantly more water than mice treated with sulfate. No differences were found in litter size, litter weaning weights, or gestational or lactational weight gain of the dams among sulfate treatments. No toxicity to the dams was found. Litters were not histopathologically examined. Fertility indices were not measured (Andres and Cline, 1989). The study is limited in that only females were treated, the number of dams per group was low and no fertility indices were measured. Moreover the pretreatment period was short.

In a 13-week feeding study of ammonium sulfate with rats, no histological changes of testes were observed up to 1792 mg/kg bw. The ovaries were not examined (see. chapter 3.1.5; Takagi et al., 1999).

Developmental Toxicity

In a gavage screening study performed according to OECD TG 422 with diammonium phosphate (250, 750, 1500 mg/kg bw in rats, animals were paired 2 weeks after start of treatment), no mortality was observed. Some treatment-related effects on hematology parameters (at 750 and 1500 mg/kg bw) were evident, and bodyweight gain was temporarily reduced in the high dose group. No treatment-related signs of clinical toxicity were observed. Banding of the enamel of the incisors of the parent animals from 750 mg/kg bw. was reported, likely due to the inhibiting effect of phosphate on mineralisation of the teeth. Offspring was unaffected by parental exposure to diammonium phosphate. Viability up to day 4 pp. and macroscopic necropsy showed no effect on the pups (The Fertilizer Institute, 2002).

In a screening study, aqueous sodium sulfate was given by gavage to 28 time-pregnant ICR mice at a dose of 2800 mg/kg bw/day from gestation days 8 through 12. The dose was selected based on previous range-finding study in non-pregnant mice, 10 % mortality was expected. Mice were allowed to deliver, and neonates were macroscopically examined, counted, and weighed on day 1 and 3. No evidence of maternal toxicity or increased resorption rate was found. The chemical had no influence on pup survival, and no adverse developmental effects on external examination were observed. When compared to controls, birth weight was significantly increased (Seidenberg,

Anderson and Becker, 1986). Fetuses were not examined histologically, and the exposure period was short compared to a valid guideline study, therefore the study is of limited reliability.

Studies in Humans

There is no data available.

Conclusion

There are no valid studies available on the effects of ammonium sulfate on fertility and development. Based on data from a similar ammonium compound (diammonium phosphate), which has been tested up to 1500 mg/kg bw in a screening study according to OECD TG 422 in rats it can be concluded that ammonium ions up to the dose tested have no negative effects on fertility. In the 13-week feeding study of ammonium sulfate with rats, no histological changes of testes were observed up to 1792 mg/kg bw. The ovaries were not examined. Fully valid studies with sulfate on fertility are not available.

In a limited study (pretreatment time short, low number of animals, no fertility indices measured) where female mice were treated with up to ca. 6550 mg sulfate/kg bw (as sodium sulfate) no effects on litter size were found.

Studies of developmental toxicity for ammonium sulfate are not available. In the screening study according to OECD TG 422 with up to 1500 mg diammonium phosphate/kg bw no effects on development have been detected in rats. In another limited screening study with exposure of mice to a single dose of 2800 mg sodium sulfate/kg bw no macroscopic effects or adverse effects on body weight gain have been detected in the pups. In both studies fetuses were not examined histopathologically.

3.2 3.2. Initial Assessment for Human Health

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Fertility and developmental toxicity studies with ammonium sulfate were not available.

As ammonium sulfate dissociates in biological systems studies with other ammonium and sulfate salts can be used to cover these endpoints: A screening study according to OECD TG 422 with ammonium phosphate as analogue substance, which forms ammonium ions in aqueous solutions is available. Fully valid fertility studies with analogue compounds containing sulfate ions are however lacking. Two limited studies with sodium sulfate can be used for assessment of fertility and developmental toxicity, however, in none of these studies have the fetuses been examined histologically. There are no in vivo data on genotoxicity for ammonium sulfate. To bridge the data gap, data for ammonium chloride, which dissociates in aqueous media to form ammonium ions, as does ammonium sulfate, will be used.

In aqueous media, ammonium sulfate dissociates in the ammonium and sulfate ions (NH_4^+, SO_4^{2-}). These can be taken up into the body by the oral and respiratory routes. Absorbed ammonium is transported to the liver and there metabolised to urea and excreted via the kidneys. Ammonium is also an endogenous substance that serves a major role in the maintenance of the acid-base balance. Minor amounts of ammonium nitrogen are incorporated in the physiological N-pool. Sulfate is a normal intermediate in the metabolism of endogenous sulfur compounds, and is excreted unchanged or in conjugated form in urine.

Ammonium sulfate is of relatively low acute toxicity (LD_{50}, oral, rat: 2000 - 4250 mg/kg bw; LD_{50} dermal, rat/mouse > 2000 mg/kg bw; 8-h LC_{50}, inhalation, rat > 1000 mg/m^3). Clinical signs after

oral exposure included staggering, prostration, apathy, and laboured and irregular breathing immediately after dosing at doses near to or exceeding the LD_{50} value. In humans, inhalation exposure to 0.1 – 0.5 mg ammonium sulfate/m³ aerosol for two to four hours produced no pulmonary effects. At 1 mg ammonium sulfate/m^3 very slight pulmonary effects in the form of a decrease in expiratory flow, in pulmonary flow resistance and dynamic lung compliance were found in healthy volunteers after acute exposure.

Neat ammonium sulfate was not irritating to the skin and eyes of rabbits.

There is no data on sensitisation available.

A 14-day inhalation study on rats exposed to 300 mg/m^3, the only tested dose, did not report histopathological changes in the lower respiratory tract. As the respiratory tract is the target organ for inhalation exposure, the NOEL for toxicity to the lower respiratory tract is 300 mg/m^3.

The NOAEL after feeding diets containing ammonium sulfate for 13 weeks to rats was 886 mg/kg bw/day. The only toxicity sign found was diarrhea in male animals of the high-dose group (LOAEL: 1792 mg/kg bw/day).

Ammonium sulfate was not mutagenic in bacteria (Ames test) and yeasts with and without metabolic activation systems. It did not induce chromosomal aberrations in mammalian or human cell cultures. No in vivo genotoxicity tests are available. Based on the negative results from in vitro studies and the negative results in the micronucleus test in vivo with ammonium chloride a mutagenic activity of ammonium sulfate in vivo is unlikely.

Similarly to other salts, high doses of ammonium sulfate may have the capability of tumor promotion in the rat stomach; it is, however, much less potent than sodium chloride when tested under identical conditions.

There are no valid studies available on the effects of ammonium sulfate on fertility and development. Based on data from a similar ammonium compound (diammonium phosphate), which has been tested up to 1500 mg/kg bw in a screening study according to OECD TG 422 in rats it can be concluded that ammonium ions up to the dose tested have no negative effects on fertility. In the 13-week feeding study of ammonium sulfate with rats, no histological changes of testes were observed up to 1792 mg/kg bw. The ovaries were not examined. Fully valid studies with sulfate on fertility are not available.

In a limited study (pretreatment time short, low number of animals, no fertility indices measured) where female mice were treated with up to ca. 6550 mg sulfate/kg bw (as sodium sulfate) no effects on litter size were found.

Studies of developmental toxicity for ammonium sulfate are not available. In the screening study according to OECD TG 422 with up to 1500 mg diammonium phosphate/kg bw no effects on development have been detected in rats. In another limited screening study with exposure of mice to a single dose of 2800 mg sodium sulfate/kg bw no macroscopic effects or adverse effects on body weight gain have been detected in the pups. In both studies fetuses were not examined histopathologically.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

The toxicity of ammonia to aquatic organisms is highly dependent on physicochemical factors, most notably pH. The acute toxicity of ammonia is also influenced to a lesser degree by temperature, carbon dioxide, dissolved oxygen, and salinity.

It is the un-ionized ammonia which is generally considered to be the primary cause of toxicity in aquatic systems. Un-ionized ammonia is more toxic to aquatic organisms than the ammonium ion because the un-ionized form is readily soluble in the lipid of the cell membrane and is rapidly absorbed by the gill. In contrast, the charged ion is not easily passed through the charged-line hydrophobic space in the membrane.

Under most environmental conditions, the un-ionized ammonia concentration is the primary driver of toxicity.

Acute Toxicity Test Results

Acute toxicity to fish

Valid tests which are suitable for determining acute toxicity to fish are available for fresh water fish species, one estuarine species, and marine species. An overview is shown in Table 3. Results for pre-adult life stages are available for four of the freshwater species, and for the marine species. All results in Table 3 are expressed in terms of $(NH_4)_2SO_4$ concentrations, though in most papers the results are explained, and in some cases concentrations are reported, in terms of ammonia (NH_3) toxicity. In all cases in which pH, temperature, and ionic strength are reported, the results are consistent with ammonia being the cause of toxicity, with increasing ratios of unionised ammonia to total ammonium with higher ambient pH and temperature.

For freshwater species, the lowest LC_{50} (96 h) value of ca. 173 mg/l is observed for juvenile *Salmo gairdneri* in a flow through test carried out at 12.4 to 12.5 °C (Thurston and Russo, 1983).

A lower LC_{50} value (10 days) of 27 mg/l is observed for one or two day post hatch larvae of the warm water marine species *Sciaenops ocellatus*, at temperatures of 25 to 26 °C and an ambient pH of 8.0 to 8.2, also in a flow through test (Holt and Arnold, 1983). Also, a substantial reduction in egg hatching success after 24 h of exposure is seen in 2250 mg/l $(NH_4)_2SO_4$, but no effect on hatching success is seen at 450 mg/l $(NH_4)_2SO_4$. An EC_{50} was not reported.

Table 3 Acute LC_{50} values for fish exposed to ammonium sulfate

Species	Habitat/ Type	Exposure Time	LC_{50} (mg/l) $(NH_4)_2SO_4$	Temp / pH	Reference
Leuciscus idus	Freshwater static	96 h	681 (geom. mean)	7.3-7.6	BASF AG, 1989a
Brachydanio rerio	Freshwater Flow through	96 h	480	23.5 °C	Larsson and Lindén, 1978
Brachydanio rerio	Freshwater Semi-static	96 h	420	23.5 °C	Larsson and Lindén, 1978
Brachydanio rerio	Freshwater Semi- static	96 h	250		ILV, 1978; Sörensen and Landner, 1978
Pimephales promelas	Freshwater static	96 h	> 100	6.5 to 8.5	Ewell et al., 1986
Salmo gairdneri	Freshwater Flow-through	96 h	ca. 173	12.4 – 12.5 °C 7.9	Thurston and Russo, 1983
Lebistes reticulatus	Freshwater Semi-static	96 h	592	18.5 – 23.5 °C 8.1 - 8.4	Chouhan and Pandey, 1987
Albumus alburnus	Estuary static	96 h	310	7.8	Lindén et al., 1979
Agonus cataphractus	Seawater static	96 h	210 (09/77) 200 (12/77) 130 (06/78)	15 °C 6.0 – 8.0	Franklin, 1980
Pimephales promelas (fresh water)	Flow through	10 days	Length and weight of young fish: NOEC = 300 LOEC = 531	20 ± 1°C 7.3	Nebeker and Schuytema, 2000
Pimephales promelas (fresh water)	Flow through	10 days	Length and weight of young fish: NOEC = 603 LOEC= 1021	20 ± 1 °C 7.2	Nebeker and Schuytema, 2000
Sciaenops ocellatus (larvae)	marine Flow-through	10 days	27	25 – 26 °C 8.0 – 8.2	Holt and Arnold, 1983
Sciaenops ocellatus (egg hatching)	marine static	24 h	NOEC = 450 LOEC = 2250	25 or 26°C 8.0 - 8.2	Holt and Arnold, 1983

Acute toxicity to aquatic invertebrates

Valid tests which are suitable for determining acute toxicity to invertebrates are available for fresh water species and marine species. An overview is shown in Table 4. All results in Table 4 are expressed in terms of $(NH_4)_2SO_4$ concentrations.

For freshwater species all LC_{50} values were > 100 mg/l.

A lower LC_{50} (96 h) of 47.7 mg/l is observed for young green mussels (*Perna viridis*), a warm water marine species, at temperatures of 28 to 30 °C and an ambient pH of 8.0 to 8.3, also in a static test (Reddy and Menon, 1979).

Table 4 Acute LC_{50} values for invertebrates exposed to ammonium sulfate

Species	**Test Type**	**Exposure Time**	**Temp/pH**	**LC_{50}, [mg $(NH_4)_2SO_4$/l]**	**Reference**
Asellus Intermedius (Crustacea)	Flow-through	96 hours	20 +/- 1 °C 6.5 - 8.5	> 100	Ewell et al. (1986)
Daphnia magna (Crustacea)	Flow-through	96 hours	20 +/- 1 °C 6.5 - 8.5	> 100	Ewell et al. (1986)
Dugesia tigrina (aquatic flatworm)	Flow-through	96 hours	20 +/- 1 °C 6.5 - 8.5	> 100	Ewell et al. (1986)
Gammarus fasciatus (Crustacea)	Flow-through	96 hours	20 +/- 1 °C 6.5 - 8.5	> 100	Ewell et al. (1986)
Helisoma trivolyis (freshwater snail)	static	48 hour 24 hours		700 (adult) 393 (juveniles)	Tchounwou, Englande and Malek (1991).
Crangon crangon (Crustacea) (seawater)	Semi-static	96 hours	15 °C 6.0 - 8.0	380 (9/77) 600 (10/77) 480 6/78 400 (9/78) 540 (3/79)	Franklin (1980)
Perna viridis (green mussel, seawater)	static	48 hours 96 hours	28 – 30 °C 8.0 - 8.3	LC_{50} = 113 LC_{50} = 95	Reddy and Menon (1979)

Acute and Chronic toxicity to algae

Only one valid test result is available which is suitable for determining toxicity to algae in freshwater systems. In long term test the green algae *Chlorella vulgaris* were exposed to ammonium sulfate for 21 days (Tam and Wong, 1996). The cell number at start time was $1*10^6$ /ml compared to about $1*10^5$ as recommended in standard guidelines like OECD TG 201. In control medium, the N-source was KNO_3, which was replaced by different concentrations of ammonium sulfate in the test media (47 - 4710 mg/l ammonium sulfate, calculated from 10 – 1000 mg/l NH_3-N). The pH value was adjusted to 7 +/- 0.2 before algae inoculation and was maintained at neutral pH during the study. In the first about 10 days after start, in all test concentration and in the control a lag phase was observed. Thereafter in the control and some test concentrations an exponential increase in cell number was observed up to day 17 or 18. No data are available to calculate the 72-h-EC_{50}.

Based on data presented in Tam and Wong (1996) an EC_{50} (18 d) for the endpoint cell count of about 2700 mg/l ammonium sulfate, but no EC_{10} can be calculated for *Chlorella vulgaris*. At concentrations of 20 - 500 mg/l N no significant differences of growth compared to control were observed.

No test according to standard guidelines is available. However, an acute toxicity effect to algae is not to be expected. The low toxicity of ammonium to algae was also found in a study with ammonium chlorid, were an EC_{50} (0 - 5 d) of 1300 mg/l was derived (OECD 2003).

Other results in Table 5 demonstrate that marine phytoplankton is more sensitive to ammonium sulfate than freshwater species, with the marine dinoflagellates *Gymnodinium splendens* and *Gonyaulax polyedra* showing growth inhibition in a 17d-test at concentrations of 0.706 mg/l ammonium sulfate (calculated from 150 µg/l NH_4-N). A reduction in photosynthesis within 3 hours is also shown for *Gymnodinium splendens* at 0.471 mg/l ammonium sulfate (calculated from 100 µg /l NH_4-N). No significance-levels are reported. A LOEC of 0.706 mg/l and a NOEC of 0.471 mg/l (calculated from 150 and 100 µg/l NH_4-N) can be estimated for the endpoint growth reduction for *Gymnodinium splendens* and *Gonyaulax polyedra*. Also, a LOEC of 0.471 mg/l and a NOEC of 0.235 mg/l (calculated from 100 and 50 µg/l NH_4-N) can be estimated for the endpoint reduction in photosynthesis for *Gymnodinium splendens*. No EC_{50} can be derived from the study. No information on pH in the test media is given (Thomas, Hastings and Fujita, 1980).

Table 5 Acute and chronic toxicity to algae

Species	**Test Type**	**Exposure Time**	**T [°C]**	**Effect [mg $(NH_4)_2SO_4$ / l]**	**Reference**
Chlorella vulgaris (algae)	Algal growth	18 days	pH: 7.0	EC_{50} = ca. 2700	Tam and Wong (1996)
Asterionella japonica (diatom) Marine phytoplankton	Growth	up to 17 days	21 - 22	NOEC > 0.942	Thomas, Hastings and Fujita (1980).
Chaetoceros affinis (diatom) Marine phytoplankton	Growth	up to 17 days	21 - 22	NOEC > 0.942	Thomas, Hastings and Fujita (1980).
Gymnodinium splendens (dinoflagellate) Marine phytoplankton	Growth Photosyn-thesis	up to 17 days 3 h	21 - 22	NOEC = 0.471 LOEC = 0.706 NOEC = 0.234 LOEC = 0.471	Thomas, Hastings and Fujita (1980).
Gonyaulax polyedra (dinoflagellate Marine phytoplankton	Growth	up to 17 days	21 - 22	NOEC = 0.471 LOEC = 0.706	Thomas, Hastings and Fujita (1980).
Dunaliella sp (rhodophyta) Marine phytoplankton	Growth	up to 17 days	21 - 22	NOEC > 0.942	Thomas, Hastings and Fujita (1980).

Chronic Toxicity Test Results

Chronic toxicity to fish

Valid tests for determining the long-term toxicity of ammonium sulfate to fish are available for three freshwater species. The results of the tests are summarized in Table 6.

For freshwater fish, the greatest sensitivity to ammonium sulfate was shown by alevins of *Oncorhynchus gorbuscha* before complete yolk absorption, with effects on the length and weight of fry at migration seen at a LOEC (61 d) of 22 mg/l $(NH_4)_2SO_4$ (calculated from 2.4 ppb NH_3). The associated NOEC (61 d) is 11 mg/l $(NH_4)_2SO_4$ (calculated from 1.2 ppb NH_3) (Rice and Bailey, 1980). Exposure of the catfish *Channa punctatus* to commercial ammonium sulfate fertilizer at 100 mg/l showed significant effect on ovarian growth, and also on testicular development, where spermatogenesis did not process beyond the spermatocyte stage, and sperm was totally absent. In this experiment correlative histological changes were also observed in the pituitary gonadotrophes (Ram and Sathyanesan, 1986, 1987).

Table 6 Toxicity of ammonium sulfate to fish in long term tests

Species	**Test Type**	**Exposure Period**	**pH**	**Temp. [° C]**	**Effect, mg $(NH_4)_2SO_4$/l**	**Reference**
Pimephales promelas (fresh water)	Flow through	10 days	7.3	20 ± 1	Length and weight of young fish: NOEC = 300 LOEC = 531	Nebeker and Schuytema, 2000
Pimephales promelas (fresh water)	Flow through	10 days	7.2	20 ± 1	Length and weight of young fish: NOEC = 603 LOEC= 1021	Nebeker and Schuytema, 2000
Oncorhynchus gorbuscha (fresh water)	Flow through	61 days	6.3-6.5	3.7-4.8	Length and weight of fry at migration. NOEC = 11 LOEC= 22	Rice and Bailey, 1980
Channa punctatus (fresh water)	Semi-static	6 months	7.2	20 to 35	Ovarian growth LOEC: 100 mg/l:	Ram and Sathyanesan, 1986
Channa punctatus (fresh water)	Semi-static	6 months	7.2		Testicular development: LOEC: 100 mg/l	Ram and Sathyanesan, 1987
Clarias batrachus	Semistatic	12 month	7		LOEC = 100 mg/l	Sathyanesan AG et al., 1978

No data is available for chronic toxicity to invertebrates.

Chronic toxicity to algae is discussed together with acute toxicity above.

Toxicity to Aquatic Species Conclusions

Freshwater Environment

Information on acute toxicity in aquatic freshwater systems is available from all three trophic levels, fish, invertebrates, and algae. Fish are the most sensitive trophic level, with the most sensitive species being juvenile *Salmo gairdneri* (Thurston and Russo, 1983), with an LC_{50} (96 h) of ca. 173 mg/l. For invertebrates, the lowest LC_{50} (24 h)of 393 mg/l is observed for juveniles of the

freshwater snail *Helisoma trivolyis* (Tchounwou, Englande and Malek, 1991). Some other results (e.g. Daphnia) are > 100 mg/l. For the algal species *Chlorella vulgaris* (Tam and Wong, 1996) an EC_{50} (18d, cell count) of ca. 2700 mg/l can be calculated.

In aquatic freshwater systems, chronic toxicity information is available for fish, the trophic level shown to be the most sensitive in acute tests. The greatest sensitivity to ammonium sulfate was shown by alevins of *Oncorhynchus gorbuscha* before complete yolk absorption, with a NOEC (61 d) of 11 mg/l $(NH_4)_2SO_4$ for effects on the length and weight of fry at emergence (Rice and Bailey, 1980). In a single concentration study at 100 mg/l, utilizing a commercial fertilizer of unspecified composition at almost an order of magnitude higher ammonium sulfate levels for 6 months, effects were seen on ovarian, testicular, and pituitary systems in the catfish *Channa punctatus* (Ram and Sathyanesan, 1986, 1987).

Marine Environment

A limited amount of ammonium sulfate toxicity data is also available for the marine environment. For acute tests the lowest LC_{50} (10 d) observed for fish is 27 mg/l ammonium sulfate for one or two day post hatch larvae of the warm water marine species *Sciaenops ocellatus* (Holt and Arnold, 1983). An invertebrate LC_{50} (96 h) of 47.7 mg/l is observed for young green mussels (*Perna viridis*), also a warm water marine species (Reddy and Menon, 1979). However, the highest toxicity in marine systems is shown by phytoplankton. The NOEC (17 d, growth inhibition) is 0.471 mg/l ammonium sulfate for the marine dinoflagellates *Gymnosium splendens* and *Gonyaulax polyedra* (Thomas, Hastings and Fujita, 1980).

Toxicity to Microorganisms

Ammonium sulfate toxicity information is available for both sewage treatment microorganisms and for microorganisms found in soil. The sewage treatment study (Suwa et al., 1994) investigated 14 strains of *Nitrobacter* spp. (ammonium oxidising bacteria) isolated from 25 different sludges including three sludges from primary sewage treatment plants and two sludges from nightsoil treatment plants. Nitrite production kinetic studies showed that insensitive strains (those which grew well at 4700 mg/l ammonium sulfate) showed Monod growth, while sensitive strains (those which grew at 94 mg/l but not at 4700 mg/l) followed Haldane kinetics. The results suggested that ammonium sulfate sensitive strains had a growth advantage in lower ammonium sulfate concentrations, while insensitive strains had a growth advantage at higher ammonium sulfate concentrations. Both sensitive and insensitive strains were found in the primary and nightsoil sludges, with the sensitive strains predominating. This explained the operational observations in several sewage treatment plants concerning the efficacy of nitrifying bacteria.

Studies of the effects of ammonium sulfate on three nitrogen-fixing soil bacteria and on total soil bacteria have also been carried out. The abundance of nitrogen-fixing cyanobacteria in a Spanish rice field was reduced significantly following a single ammonium sulfate application even at the lowest application of 82.5 kg/ha (calculated from 17.5 kg N/ha) (Fernández Valiente et al., 2000). In another experiment, biological nitrogen-fixing ability in a field under crop rotation in southern Sweden was reduced (nitrogen-fixing legume bacteria) or eliminated (nitrogen-fixing blue green algae) by over thirty years annual application of 377 kg/ha ammonium sulfate (calculated from 80 kg N/ha) (Martensson and Witter, 1990). The lowering of soil pH by ammonium sulfate was the main cause of the reduction in the nitrogen-fixing capacity of the soil. In this experimental field, total soil biomass was reduced by almost 50 % relative to unfertilized control plots (Witter, Martensson and Garcia, 1993), although base respiration rate was unaffected.

Conclusion

Nitrification during sewage treatment plant operation involves both sensitive (no growth at 4700 but growth at 94 mg/l ammonium sulfate) and insensitive (growth at 4700 mg/l ammonium sulfate) strains of *Nitrobacter* spp (Suwa et al., 1994). This indicates that a NOEC for specific nitrifying bacteria will be greater than 94 mg/l.

Nitrogen fixation and total soil biomass (but not soil base respiration rate) can be affected by ammonium sulfate applied at 82.5 kg/ha or more.

4.2 Terrestrial Effects

Toxicity to terrestrial plants

Valid test results are available for four plant species exposed to excessive amounts of ammonium sulfate. In two examples ammonium sulfate is applied in solid form, while in the others the application is from aqueous solution and as an atmospheric aerosol.

The effect of ammonium sulfate solution on seed germination was studied for *Avena sterilis* spp *macrocarpa* Mo, in a 21 day test (Gonzalez Ponce and Salas, 1989). Seeds were wrapped with filter paper which was wetted with ammonium sulfate-solutions of 100 to 5000 mg/l. No significant increase in germination was found up to 2500 mg/l, compared with the control. An inhibitory effect was found at 5000 mg/l ammonium sulfate probably caused by a salt effect.

The effect of ammonium sulfate addition on the growth of the onion *Allium cepa* L. has been studied under laboratory conditions in 4 Canadian soils, in the presence of lime to raise the soil pH to approximately 6.5 (Abbés et al., 1995). After 84 days in a growth chamber, immature plants were harvested and fresh and dry weight of all plant parts were determined. Yield was greatest for 626 mg ammonium sulfate / kg soil (calculated from 133 mg N / kg soil). An inhibitory effect was found at 1880 and 2506 mg ammonium sulfate / kg soil (calculated from 399 and 532 mg N / kg soil), except for the sandy soil where only 2506 mg ammonium sulfate / kg soil was inhibitory. In general such observations could be explained by salt effects.

14 day old pinto bean plants (*Phaseolus vulgaris* L.) exposed to 26 mg/m^3 ammonium sulfate aerosol for up to 320 hours (ca. 13 days) in an environmental growth chamber showed no changes in plant biomass or leaf area. However, visible foliar injury occurred, and both abaxial and adaxial leaf resistances were decreased from control values (Gmur, Evans and Cunningham, 1983). The ammonium sulfate application rate is stated to be about 2 orders of magnitude above ambient episode concentration.

The 6 year effect of ammonium sulfate spread as a solid fertilizer was investigated in a stand of trees (*Picea abies*, 12 years old at the beginning of the test) in southern Sweden (Rosengren-Brinck and Nihlgard, 1995). Spreading at 471 kg ammonium sulfate (calculated from 100 kg N) per ha per year affected resistance to drought, which was evident in a reduction in the flushing of new shoots.

Toxicity to Soil Dwelling Organisms

Valid test results are available for two soil dwelling species, Collembola and Cryptostigmata (family Acarina, mites) (Heneghan and Bolger, 1996). Ammonium sulfate, simulating acid rain, was deposited in monthly applications at total amount of 708 kg ammonium sulfate/ha/year to a field planted with *Picea abies*. The soil fauna was extracted and counted twice, at the end of years 1 and 2. In the first year evaluation, a significant increase of the numbers of Cryptostigmata in the organic layer to more than double of control level was detected. In the second year, the abundances of Cryptostigmata were still high, but the differences to control were not significant. On both occasions the numbers of Collembola in the organic and top 6 cm mineral layers were significantly increased relative to controls.

Conclusion

In plants applications of 471 kg ammonium sulfate per ha per year for 6 years affects drought resistance in *Picea abies*. This effect can be explained by salt effects. The soil fauna is less sensitive, with both Collembolla and Cryptostigmata numbers increasing with 708 kg ammonium sulfate application per ha per year.

4.3 Other Environmental Effects

Toxicity to Amphibians

Information is available on the toxicity of ammonium sulfate to the amphibians *Pseudacris regilla* (Pacific treefrog), *Ambystoma gracile* (Northwestern salamander), and *Rana aurora* (Redlegged frog) (Nebeker and Schuytema, 2000). All tests were carried out for 10 days, in aquaria with a continuous flow of test solution.

Two experiments were carried out with *Pseudacris regilla* tadpoles from different egg masses, aged 6 and 9 weeks at the start of the test. Endpoints were growth and wet weight. The NOEC for the 6 week-old tadpoles was 82 mg/l ammonium sulfate (calculated from 17.4 mg/l NH_4-N). The corresponding LOEC was 154 mg/l ammonium sulfate (calculated from 37.0 mg/l NH_4-N). The NOEC for the 9 week-old tadpoles was 153 mg/l ammonium sulfate (calculated from 32.4 mg/l NH_4-N). The corresponding LOEC was 247 mg/l ammonium sulfate (calculated from 52.5 mg/l NH_4-N). The LC_{50} (10 d) for both 6 and 9 weeks old *Pseudacris regilla* tadpols was > 995 mg/l (calculated from 211.2 mg/l NH_4-N).

Larvae of the Northwestern salamander (*Ambystoma gracile*) were 5 weeks old at the beginning of the test. The NOEC for the endpoint wet weight was 384 mg/l ammonium sulfate (calculated from 81.5 mg/l NH_4-N). The corresponding LOEC was 596 mg/l ammonium sulfate (calculated from 126.5 mg/l NH_4-N). The LC_{50} (10 d) for *Ambystoma gracile* was > 995 mg/l (calculated from 211.2 mg/l NH_4-N).

Tadpoles of the Redlegged frog (*Rana aurora*) were 4 weeks old at the beginning of the test. The NOEC for for both endpoints growth and wet weight was 390 mg/l ammonium sulfate (calculated from 82.7 mg/l NH_4-N). The corresponding LOEC was 631 mg/l ammonium sulfate (calculated from 134.0 mg/l NH_4-N). The LC_{50} (10 d) for *Rana aurora* was > 995 mg/l (calculated from 211.2 mg/l NH_4-N).

Beetles of the species *Thermonactus basillaris* were treated with aerial application of 23.52 and 35.29 kg ammonium sulfate /ha, respectively. After 24 h a Motality of 4.4 % was observed in the higher application group. No mortality occurred at the lower application group (Apgar, 1985).

Hen: a reduction in weight gain and gain/feed ratio was examined after 14 days in chicken fed with ammonium sulfate when the concentration in food exceeded 10 000 mg/kg food. The validity of the result cannot be assessed because of insufficient documentation of the study (Sibbald, 1976).

Conclusion

The most sensitive amphibians were the 6 week-old *Pseudacris regilla* tadpoles, with a NOEC (growth, wet weight) of 82 mg/l ammonium sulfate. The corresponding LOEC was 154 mg/l ammonium sulfate. The LC_{50} (10 d) for 6 and 9 weeks old *Pseudacris regilla*, for *Rana aura* and *Ambystoma gracile* was > 995 mg/l.

4.4 Initial Assessment for the Environment

Ammonium sulfate is a white solid, with a solubility in water of 764 g/l at 25 °C. When heated, decomposition starts at temperatures between 150 and 280 °C, depending on the experimental conditions and purity of the test substance, and is complete at 336 - 357 °C. The relative density is 1.77, and the partial pressure of ammonia over solid ammonium sulfate at 25 °C is $4.053*10^{-7}$ Pa. The log K_{OW} was determined as –5.1 in a test according to OECD TG 107; as this method applies only to substances which do not dissociate, the validity of this method for ammonium sulfate is uncertain. Due to the ionic nature of the substance the calculation of sorption onto organic soil matter does not have any practical meaning.

Due to the salt-character of the substance the calculation of a fugacity model and Henrys Law Constant is not appropriate. Based on the physico-chemical properties of ammonium sulfate, water is expected to be the main target compartment.

Although ammonium sulfate does not volatilize, it can, especially if applied on the soil surface, decompose in soil to release ammonia, which will volatilize. Although ammonium sulfate can be created in the atmosphere from ammonia and sulfur dioxide, this process is limited by atmospheric sulfur dioxide, not by ammonia, which has many natural sources. Particulate ammonium sulfate is removed from air by wet and dry deposition. There is no evidence for photodegradation of ammonium sulfate.

In unsterilized soil, ammonium sulfate is mineralized fairly rapidly, and subsequently nitrified. Nitrification and de-nitrification processes also occur naturally in streams and rivers, as well as in many secondary sewage treatment processes. Based on high water solubility and the ionic nature, ammonium sulfate is not expected to adsorb or bioaccumulate to a significant extent. However, mobility in soil may be reduced through ion-ion interactions.

Environmental effects can be assessed in the freshwater and marine environments. In addition, some information is available for soil and sewage treatment micro-organisms, for freshwater sediment, and for the terrestrial environment.

Freshwater Environment

The lowest acute and chronic toxicity values for the three trophic levels for which freshwater ammonium sulfate data is available are shown in Table 7.

Table 7 Data used in the Freshwater PNEC determination of Ammonium sulfate

Test Type	Trophic Level	Species	Result
Acute	Fish	juvenile *Salmo gairdneri*	LC_{50} (96h) = 173 mg/l
Acute	Invertebrates	juvenile freshwater snail *Helisoma trivolyis*	LC_{50} (24h) = 393 mg/l
Acute	Invertebrates	*Daphnia magna*	EC_{50} (96h) > 100 mg/l
Acute	Aquatic Plants	*Chlorella vulgaris*	LC_{50} (18d) > 2700 mg/l (cell count)
Chronic	Fish	alevins of *Oncorhynchus gorbuscha*	NOEC (61d) = 11 mg/l

The PNEC for the freshwater aquatic environment is based upon the lowest observed chronic toxicity result, the NOEC value of 11 mg/l ammonium sulfate for alevins of *Oncorhynchus gorbuscha*. An assessment factor of 100 is appropriate, leading to a freshwater aquatic PNEC of 0.11 mg/l.

Supporting information is also available for amphibians. The most sensitive amphibians were 6 week-old *Pseudacris regilla* tadpoles, with a NOEC (10d) of 82 mg/l ammonium sulfate.

Marine Environment

Marine acute data are available for fish, invertebrates and for phytoplankton, the latter being most sensitive. For *Gymnodinium splendens*,and *Gonyaulax polyedra* growth reduction was found at concentrations of 0.7 mg/l and above. No EC_{50} value can be derived. For seawater invertebrates the lowest effect value was obtained for green mussel *Perna viridis* (96 h-LC_{50} = 47.7 mg/l). For marine fish the lowest effect value was found for larvae of *Sciaenops ocellatus* with a LC_{50} (10 d) of 27 mg/l.

Micro-organisms in sewage treatment

Nitrification during sewage treatment plant operation involves both sensitive (no growth at 4700 but growth at 94 mg/l ammonium sulfate) and insensitive (growth at 4700 mg/l ammonium sulfate) strains of *Nitrobacter spp*. These results indicate that a NOEC for specific nitrifying bacteria will be greater than 94 mg/l.

Terrestrial Environment

The results of the terrestrial plant and soil bacteria studies show that the major effect of repeated ammonium sulfate application is a reduction in soil pH, which in agricultural situations is controlled by liming. The most toxic results for specific soil bacteria, for cyanobacteria in rice fields, show less than 50 % reduction in nitrogen fixation at 82.5 kg/ha/year in the absence of liming. Similar results are seen for plants, with 471 kg ammonium sulfate per ha per year for 6 years affecting drought resistance in (*Picea abies*). The soil fauna is less sensitive, with both *Collembolla* and *Cryptostigmata* numbers increasing under 708 kg/ha/yr ammonium sulfate application.

5 RECOMMENDATIONS

Environment:

The chemical possesses properties indicating a hazard for the environment. These hazards do not warrant further work, as they are related to acute toxicity which may become evident only at high exposure levels, they should nevertheless be noted by chemical safety professionals and users..
Although the substance has a low inherent hazard potential for the environment, it degrades in the environment to nitrite. It is recommended that the use of ammonium sulfate as a fertilizer is taken into account when assessing the exposure of nitrite and nitrate to humans through drinking water. The chemical is currently of low priority for further work.

Human Health:

The chemical is currently of low priority for further work because of its low hazard potential.

6 REFERENCES

Abbés LE, Parent A, Karam A, and Isfan D (1995). Onion response to ammoniated peat and ammonium sulfate in relation to ammonium toxicity. Canadian J Soil Sci. 75, 3, p 261-272.

Amdur MO, Bayles J, Ugro V, and Underhill DW (1978). Comparative irritant potency of sulfate salts. Environm. Res. 16, 1-8.

Andres CJ and Cline TR (1989). Influence of sulfate in drinking water on mouse reproduction during two parties. J. Anim. Sci. 67, 1313-1317.

Avol EL, Jones MP, Bailey RM, Chang N-MN, Kleinman MT, Linn WS, Bell KA and Hackney JD (1979). Controlled Exposures Of Human Volunteers To Sulfate Aerosols Health Effects And Aerosol Characterization. Am. Rev. Respir. Dis. 120, 319-327.

BASF AG (1968). Department of Toxicology. Unpublished data (XVII/352-363), 24 January 1968.

BASF AG (1969). Department of Toxicology. Unpublished data (XIX/179), 09 September 1969.

BASF AG (1988). Department of Analytics. Unpublished study. (BRU 88.222).

BASF AG (1989a). Department of Toxicology. Unpublished report (88/736). 27 April 1989.

BASF AG (1989b). Department of Toxicology. Unpublished report (88/736). 26 June 1989.

BASF AG (1999). Material Safety Data Sheet. Ammonium Sulfat 21 % N grob. 27 December 1999.

BASF AG (2002). Product Information “Ammonium sulfate industrial grade”, October 2002.

BASF AG (2003a). Internal Communication, 06.03.2003.

BASF AG (2003b). Internal Communication, 25.03.2003.

BASF AG (2003c). Internal Communication, 09.05.2003.

BASF AG (2004). Workplace measurements according GefSoffV. § 18, 24/25.June 2004, Project. No: DE/LU/L515/0267/04, 30. June 2004.

Berden M and Nilsson SI (1996). Influence of added ammoniumsulfate on the leaching of aluminium, nitrate, and sulfate- a laboratory experiment. Water, Air, and Soil Pollution 87, 1-22.

Budavari S (1996). The Merck Index. Whitehouse Station. Merck Research Laboratories.

Busch RH, Buschbom RL, Cannon WC, Lauhala KE, Miller FJ, Graham JA, and Smith LG (1984). Effects of ammonium sulfate aerosol exposure on lung structure of normal and elastase-impaired rats and guinea pigs. Environm. Res. 33, 2, 454-472.

Carnol M, Ineson P, and Dickinson ML (1997). Soil solution nitrogen and cations influenced by $(NH_4)_2SO_4$ deposition in a coniferous forest. Environmental Pollution 97, 1-2, 1-10.

Carnol M, Ineson P, Anderson JM, Beese F, Berg MP, Bolger T, Coûteaux MM, Cudlin P, Dolan S, Raubuch M, and Verhorf HA (1997). The effects of ammonium sulfate deposition and root sinks on soil solution chemistry in coniferous forest soils. Biogeochemistry 38, 255-280.

Chouhan MS and Pandey AK (1987). On the tolerance limits and toxicity symptoms of guppy (*Lebistes reticulatus*) to fertilizers: urea and ammonium sulfate. Comp. Physiol. Ecol. 12, 77-80.

Christen HR (1973). Grundlagen der allgemeinen und anorganischen Chemie, 4., neu bearbeitete Auflage, herausgegeben von Sauerländer AG, Aarau und J. Fink, Stuttgart, S. 354.

CRC (2002). Handbook of Chemistry and Physics, CRC Press, 83rd edition.

Danish Product Register (2002). Communication to BUA.

David M, Vance G, and Kahl J (1999). Chemistry of dissolved Organic carbon at bear Brook Watershed, Maine: Stream Water Response to (NH4)2SO4 Additions. Environmental Monitoring and Assessment 55, 149-163.

Debreczeni K and Berecz K (1998). Monitoring of gaseous nitrogen losses from nitrogen fertilizers in model experiments. Commun. Soil Sci. Plant Anal. 29, 2207-2216.

EPA (1978). U.S. Environmental Protection Agency. Studies on the Effect of Ammonium Sulfate on Carcinogenesis. March 1978. EPA-600/1-78-020, National Technical Information Service Publication PB 280 336.

EPA (2002). U.S. Environmental Protection Agency. Drinking Water Advisory: Consumer Acceptability Advice and Health Effects Analysis on Sulfate (Draft). April 2002.

EU (1995). European Parliament and Council Directive No 95/2/EC of 20 Feb 1995 on food additives other than sweeters, (OJL61, 18.3., 1995, p1, Annex V), amended by Directive 2003/52/EC of European Parliament and Council of 18 June 2003, p23, 17.7. 2003.

European Environmental Agency (2004). http://dataservice.eea.eu.int/dataservice.

Ewell WS, Gorsuch JW, Kringle RO, Robillard KA, and Spiegel RC (1986). Simultaneous Evaluation of the Acute Effects of Chemicals on Seven Aquatic Species. Environm. Toxicol. Chem. 5, 831-840.

FDA (2003). Code of Federal Regulations: Food and Drugs Part 182 and 184, Office of the Federal Register. National Archives and Records Administration. Washington, DC.

Fernández Valiente E, Ucha A, Quesada A, Leganés F, and Carreres R (2000). Contribution of N_2 fixing cyanobacteria to rice production: availability of nitrogen from 15N-labelled cyanobacteria and ammonium sulfate to rice. Plant Soil 221, 107-112.

Frank R, Morgan MS, Koenig JQ, Horike N, Covert DS, and Holub R (1977). Respiratory effects of inhaled gases and aerosols. Rept. for 1 Nov 73 - 31 Mar 77. Washington Univ., Seattle, Dept. of Environmental Health. Jul 77, 57 p, prepared for NIOSH, Rockville, MD, NTIS/PB89-227862.

Frank R (1980). Coal Consumption, Ambient Air Pollution and the Lung. Health Implic New Energy Technol (Park City Environ Health Conf), 347-358.

Franklin F (1980). Assessing the Toxicity of Industrial Wastes, with Particular Reference to Variations in Sensitivity to Test Animals. Fisheries Research Technical Report Number 61. Ministry of Agriculture, Fisheries and Food.

Furihata C, Yamakoshi A, Takezawa R, and Matsushima T (1989). Various Sodium Salts, Potassium Salts, a Calcium Salt and an Ammonium Salt Induced Ornithine Decarboxylase and Stimulated DNA Synthesis in Rat stomach Mucosa. Jpn. J. Cancer Res. 80, 424-429.

Gonzalez Ponce R, and Salas ML (1989). Effects of different nitrogen compounds and temperatures on the germination of *Avena sterilis spp Macrocarpa* Mo. Biologia Plantarum (Praha), **31,** 4, 261-268.

Gmelin (1936). Gmelins Handbuch der anorganischen Chemie. Ammoniumsulfat. Vol. 23, p. 261-268.

Gmur NF, Evans LS, and Cunningham EA (1983). Effects of ammonium sulfate aerosols on vegetation - II. Mode of entry and responses of vegetation. Atmospheric Environm. 17, 715-721.

Godleski JJ, Melnicoff MJ, Sadri S, and Garbeil P (1984). Effects of inhaled ammonium sulfate on benzo(a)pyrene carcinogenesis. J. Toxicol. Environm. Health 14, 225-238.

Hayashi M, Kishi M, Sofuni T, and Ishidate JRM (1988). Micronucleus test in mice on 39 food additives and eight miscellaneous chemicals. Food. Chem. Toxicol. 26, 487-500.

Heneghan L and Bolger T (1996). Effects of acid rain components on soil microarthropods: a field manipulation. Pedobiologica 40, 413-438.

Holt GJ and Arnold CR (1983). Effects of Ammonia and Nitrite on Growth and Survival of Red Drum Eggs and Larvae. Trans. Am. Fish. Soc. 112, 314-318.

HSDB (2002). HSDB Data Bank, through 2002/10.

IFIA (2002). International Fertilizer Industry Association, “Ammonium Sulfate Statistics 2002”, Paris (France).

ILV (1978). Inst. foer Vattn- och Luftvardsforskning, Stockholm, Sweden, report B 425.

Johnson WW and Sanders HO (1977). Chemical forest fire retardants. Acute toxicity to five freshwater fishes and ascud. Technical Papers of the U.S. Fish and Wildlife Service.

Kirk-Othmer (1984). Kirk-Othmer Encyclopedia of Chemical Technology. 3rd ed. Volumes 1-26, New York, NY. John Wiley and Sons, 1978-1984, p. S 444. In: HSDB (2002).

Koenig JQ, Dumler K, Rebolledo V, Williams PV, and Pierson WE (1993). Respiratory effects of inhaled sulphuric acid on senior asthmatics and nonasthmatics. Arch. Environm. Health 48, 171-175.

Kulle TJ, Sauder LR, Shanty F, Kerr HD, Farrell BP, Miller WR, and Milman JH (1984). Sulfur dioxide and ammonium sulfate effects on pulmonary function and bronchial reactivity in human subjects. Am. Ind. Hyg. Assoc. J. 45, 156-161.

Larsson A and Lindén E.(1978). Results and Experiences of the "Iso Ringtet" on Determination of Acute toxicity to Fish. Nordfolk Miljoevardssekr. Publ. 2, 303-312.

Leaderer BP, Naeher L, Jankun Th, Balenger K, Holford TR, Toth C, Sullivan J, Wolfson JM, and Koutrakis P (1999). Indoor, Outdoor, and regional summer and winter concentrations of PM10 , PM25, SO_4^{2-}, H^+, NH_4^+, NO_3^-, NH_3, and nitrous acid in homes with and without kerosene space heaters. Environm. Health Perspect. 107, 223-231.

Lindén E, Bengtsson B-E, Svanberg O, and Sundström 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.

Litton Bionetics (1975). Mutagenic evaluation of compound FDA 73-42: Ammonium sulfate granular food grade. Submitted to the US Food and Drug Administration, 30 June 1975. National Technical Information Service PB, 245-506.

Loscutoff SM, Cannon WC, Buschbom RL, Busch RH, and Killand BW (1985). Pulmonary function in elastase-treated guinea pigsand rats exposed to ammonium sulfate or ammonium nitrate aerosols. Environm. Res. 36, 170-180.

Martensson M and Witter E (1990). Influence of various soil amendments on nitrogen-fixing soil microorganisms in a long term field experiment, with special reference to sewage sludge. Soil Biol. Biochem. 22, 977-982.

Moal JF, Martinez J, Marol C, and Guiraud G (1994). A direct incorporation of N-15 labelled ammonium sulfate into a pig slurry: a laboratory experiment on NH3 volatilization. Bioresource Technol. 48, 87-89.

Nadelhoffer K, Downs M, Fry B, Magill A, and Aber J (1999). Controls on N retention and exports in a forested watershed. Environm. Monitoring Assessment 55, 187-210.

Nebeker AV and Schuytema GS (2000). Effects of Ammonium Sulfate on Growth of Larval Northwestern Salamanders, Red-Legged and Pacific Treefrog Tadpoles, and Juvenile Fathead Minnows. Bull. Environ. Contam. Toxicol. 64, 271-278.

Obe G, Jonas R, and Schmidt S (1986). The restrictionendonuclease Alu I induces chromosomal aberrations in humanperipheral lymphocytes in vitro. Mutat. Res. 163, 271-275.

OECD (2003). SIDS Initial Assessment Profile: Ammonium chloride (12125-02-9).

Pepelko WE, Mattox JK, and Cohen AL (1980). Toxicology of ammonium sulfate in the lung. Bull. Environm. Contam. Toxicol. 24, 156-160.

Perrin TS, Boettinger JT, Frost DT, and Norton JM (1998). Decreasing Nitrogen leaching from sandy soil with ammonium-loaded clinoptilolite. J. Environ. Qual. 27, 656-663.

Phalen RF, Kengyer, Crocker TT, and McClure TR (1980). Effects of sulfate aerosols in combination with ozone on elimination of tracer particles inhaled by rats. J. Toxicol. Environm. Health 6, 797-810.

Ram RN and Sathyanesan AG (1986). Ammonium sulfate induced nuclear changes in the oocyte of the fish, Channa punctatus (Bl.). Bull. Environ. Contam. Toxicol. 36, 871-875.

Ram RN and Sathyanesan AG (1987). Effect of chronic exposure of commercial nitrogenous fertilizer, ammonium sulfate, on testicular development of a teleost Channa punctatus (Bloch). Indian J. Exp. Biol. 25, 667-670.

Reddy NA and Menon NR (1979). Effects of ammonia and ammonium on tolerance and byssogenesis in *Perna viridis*. Marine Ecol. Progr. Ser. 1, 315-321.

Rice SD and Bailey JE (1980). Survival, size, and emergence of pink salmon, *Oncorhynchus gorbuscha*, alevins after short- and long-term exposures to ammonia. Fishery Bull. 78, 641-648.

Rosengren-Brinck U and Nihlgard B (1995). Effects of nutritional status on the drought resistance in Norway spruce. Water, Air, Soil Pollution 85, 1739-1744.

Sackner MA, Dougherty RL, Chapman GA, Cipley J, Perez D, Kwoka M, Reinhart M, and Brito M (1981). Effects of brief and intermediate exposures to sulfate submicron aerosols and sulfate injections on cardiopulmonary function of dogs and tracheal mucous velocity of sheep. J. Toxicol. Environm. Health 7, 951-972.

Sato A, Gonmori K, and Yoshioka N (1999). A case of fatal intoxication with ammonium sulfate and a toxicological study using rabbits. Forensic Sci. Int. 101, 141-149.

Schlesinger RB (1984). Comparative Irritant Potency of Inhaled Sulfate Aerosols - Effects on Bronchial Mucociliary Clearance. Environm. Res. 34, 268-279.

Schlesinger RB (1989). Factors affecting the response of lung clearance systems to acid aerosols: role of exposure concentration, exposure time, and relative acidity. Environm. Health Perspect. 79, 121-126.

Scott WD and Cattell FCR (1979). Vapor Pressure of Ammonium Sulfates. Atmospheric Environm. 13, 307-317.

Seidenberg JM, Anderson DG, and Becker RA (1986). Validation of an in Vivo Developmental Toxicity Screen in the Mouse. Teratogen. Carcinogen Mutagen. 6, 361-374.

Shahandeh H, Cabrera L and Sumner ME (1992). Ammonia Volatilization from Urea, ammonium sulfate, and Nutrasweet sludge in bare and straw covered soils. Commun. Soil Sci. Plant Anal. 23, 7 & 8, 775-786.

Sinclair JD, Psota-Kelty LA, Peins GA, and Ibidunni AO (1992). Indoor/outdoor relationships of airborne ionic substances: comparison of electronic equipment room and factory environments. Atmospheric Environm. 26A, 5, 871-882.

Smith LG, Busch RH, Buschbom RL, Cannon WC, Loscutoff SM, and Morris JE (1989). Effects of sulfur dioxide or ammonium sulfate exposure, alone or combined, for 4 or 8 months on normal and elastase-impaired rats. Environm. Res. 49, 60-78.

Sörensen L and Landner L (1978). The ISO Ringtest ondetermination of acute toxicity to fish. Institut för Vatten- och Luftvardsforshkning. February, 1978.

Stacy RW, Seal Jr E, House DE, Green J, Roger LJ, and Raggio L (1983). A survey of effects of gaseous and aerosol pollutants on pulmonary function of normal males. Arch. Environm. Health 38, 104-115.

Suwa Y, Imamura Y, Suzuki T, Tashiro T, and Urushigawa Y (1994). Ammonia-Oxidizing bacteria with different sensitivities to (NH4)2SO4 in activated sludges. Wat. Res. 28, 1523-1532.

Sveda R, Rechcigl JE, and Nkedi-Kizza P (1992). Evaluation of various nitrogen sources and rates on nitrogen movement, Pensacola bahiagrass production, and water quality. Commun. Soil Sci. Plant Anal. 23, 17-20, 2451-2478.

Swaen GM, Slangen JMM, Ott MG, Kusters E, Van Den Langenbergh G, Arends JW, and Zober A (1996). Investigation of a cluster of ten cases of Hodgkin`s disease in an occupational setting. Int. Arch. Occup. Environ. Health 68, 224-228.

Swedish Product Register (2002). Communication to BUA.

Swiss Product Register (2002). Communication to BUA.

Tam NFY and Wong YS (1996). Effect of ammonia concentrations on growh of Chlorella vulgaris and nitrogen removal from media. Bioresource Technol. 57, 45-50.

The Fertilizer Institute (2002). Unpublished data. DAP.4-Week General Toxicity and Reproductive/Developmental Toxicity Screening Test. WBG 001/022948. 20 November 2002.

Takagi H, Onodera H, Yun L, Yasuhara K, Koujitani T, Mitsumori K, and Hirose M (1999). 13-Week Subchronic Oral Toxicity Study of Ammonium Sulfate in Rats. Bull. Natl. Health Sci. 117, 108-114 [English translation].

Tchounwou PB, Englande AJ, and Malek EA (1991). Toxicity evaluation of ammonium sulfate and urea to three developmental stages of freshwater snails. Arch. Environ. Contam. Toxicol. 21, 359-364.

Thomas WH, Hastings J, and 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. Marine Environm. Res. **3**, 291-296.

Thurston RV and Russo RC (1983). Acute toxicity of ammonia to rainbow trout. Trans. Am. Fish. Soc. 112, 696-704.

Tuschy S and Obe G (1988). Potentiation of Alu I-induced chromosome aberrations by high salt concentrations in Chinese hamster ovary cells. Mutat. Res. 207, 83-87.

UBA (2003). Environmental Data Germany 2002.

Ullmann (2000). Ullmann's Encyclopedia of Industrial Chemistry, Sixth Edition, 2000 Electronic Release.

Utell MJ, Morrow PE, and Hyde RW (1982). Comparison of normal and asthmatic subjects' responses to sulphate pollutant aerosols. Ann. Occup. Hyg. 26, 691-697.

Utell MJ, Morrow PE, Speers DM, Darling J, and Hyde RW (1983). Airway responses to sulfate and sulfuric acid aerosols in asthmatics: An exposure-response relationship. Am. Rev. Respir. Dis. 128, 444-450.

Weijers EP and Vugts HF (1990). The composition of bulk precipitation on a coastal island with agriculture, compared with an urban region. Atmospheric Environment 24A, 3021-3031.

WHO (1986). Environmental Health Criteria 54. Ammonia. Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organization. Geneva, 1986.

WHO (1996). Guidelines for drinking-water quality, 2nd ed. Vol. 2. Health criteria and other supporting information. Geneva

Witter E, Martensson AM and Garcia FV (1993). Size of the soil microbial biomass in a long-term field experiment as affected by different N-fertilizers and organic manures. Soil Biol. Biochem. **25**, 659-669.

Yamanaka S, Hashimoto M, Tobe M, Kobayashi K, Sekizawa J, and Nishimura M (1990). A simple method for screening assessment of acute toxicity of chemicals. Arch. Toxicol. **64**, 262-268.

I U C L I D

D a t a S e t

Existing Chemical	ID: 7783-20-2
CAS No.	7783-20-2
EINECS Name	ammonium sulphate
EC No.	231-984-1
Molecular Weight	132.14 g/mol
Molecular Formula	H8 N2 O4 S

Producer Related Part

Company:	BASF AG
Creation date:	12-NOV-1992

Substance Related Part

Company:	BASF AG
Creation date:	12-NOV-1992

Memo:	master
Printing date:	31-JUL-2006
Revision date:	
Date of last Update:	18-APR-2006
Number of Pages:	231
Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, SIDS

1.0.1 Applicant and Company Information

Type: lead organisation
Name: BASF AG
Contact Person: Dr. Rolf Sarafin
Date:
GUP/CR - Z570
Street: Carl-Bosch-Strasse
Town: 67056 Ludwigshafen
Country: Germany
Phone: +49 621 60 44712
Telefax: +49 621 60 58043
Email: rolf.sarafin@basf.com
Homepage: www.basf.com

Flag: Critical study for SIDS endpoint
10-APR-2006

Type: cooperating company
Name: Bayer AG
Country: Germany

Flag: Critical study for SIDS endpoint
11-JUL-2003

Type: cooperating company
Name: DSM NV
Country: Netherlands

Flag: Critical study for SIDS endpoint
11-JUL-2003

Type: cooperating company
Name: Honeywell
Country: United States

Flag: Critical study for SIDS endpoint
11-JUL-2003

Type: cooperating company
Name: Ineos Acrylics Inc.
Country: United Kingdom

Flag: Critical study for SIDS endpoint
11-JUL-2003

Type: cooperating company
Name: JFE Chemical Corp.
Country: Japan

Flag: Critical study for SIDS endpoint
31-JUL-2003

Type: cooperating company
Name: Kuraray Co., Ltd.
Country: Japan

Flag: Critical study for SIDS endpoint
11-JUL-2003

Type: cooperating company
Name: Mitsubishi Chemical Corporation
Country: Japan

Flag: Critical study for SIDS endpoint
11-JUL-2003

Type: cooperating company
Name: Nakayama Steel Works Ltd.
Country: Japan

Flag: Critical study for SIDS endpoint
11-JUL-2003

Type: cooperating company
Name: Nippon Steel Chemical Co., Ltd.
Country: Japan

Flag: Critical study for SIDS endpoint
11-JUL-2003

Type: cooperating company
Name: Sumika Agrotech Co., Ltd.
Country: Japan

Flag: Critical study for SIDS endpoint
11-JUL-2003

Type: cooperating company
Name: Sumikin Chemical Co., Ltd.
Country: Japan

Flag: Critical study for SIDS endpoint
11-JUL-2003

Type: cooperating company
Name: Sumitomo Chemical Co., Ltd.
Country: Japan

Flag: Critical study for SIDS endpoint
11-JUL-2003

Type: cooperating company
Name: Toray Industries, Inc.
Country: Japan

Flag: Critical study for SIDS endpoint
11-JUL-2003

Type: cooperating company
Name: Ube Industries, Ltd.
Country: Japan

Flag: Critical study for SIDS endpoint
11-JUL-2003

1.0.2 Location of Production Site, Importer or Formulator

1.0.3 Identity of Recipients

1.0.4 Details on Category/Template

1.1.0 Substance Identification

Mol. Formula: $H_8N_2O_4S$
Mol. Weight: 132.14 g/mol

Flag: non confidential, Critical study for SIDS endpoint
23-MAY-2003

1.1.1 General Substance Information

Substance type: inorganic
Physical status: solid
Colour: white

Remark: Form: crystalline
Flag: non confidential, Critical study for SIDS endpoint
11-JUL-2003 (1)

Substance type: inorganic
Physical status: solid
Colour: waterclear

Remark: not hygroscopic
pungent salty tasting
Flag: non confidential, Critical study for SIDS endpoint

26-MAY-2003 (2)

Purity: >= 99 - % w/w

Method: titration
Test substance: Ammonium Sulfate Industrial Grade
Flag: non confidential, Critical study for SIDS endpoint
11-JUL-2003 (3)

1.1.2 Spectra

1.2 Synonyms and Tradenames

Ammonium sulfate

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Ammonium sulfate ((NH4)2SO4)

Flag: non confidential, Critical study for SIDS endpoint

02-DEC-1992

Ammonium sulphate

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Ammoniumsulfat

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Diammonium sulfate

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Diammonium sulphate

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Mascagnite

Flag: non confidential, Critical study for SIDS endpoint
26-JAN-2004

Sulfuric acid ammonium salt (1:2)

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Sulfuric acid diammonium salt (8CI, 9CI)

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

Sulfuric acid, diammonium salt

Flag: non confidential, Critical study for SIDS endpoint
02-DEC-1992

1.3 Impurities

CAS-No: 7439-89-6
EC-No: 231-096-4
EINECS-Name: iron
Mol. Formula: Fe

Method: photometry
Remark: <= 5 mg/kg (ppm)
Test substance: Ammonium Sulfate Industrial Grade
Flag: non confidential, Critical study for SIDS endpoint
11-JUL-2003 (3)

EINECS-Name: heavy metals (sum)

Method: colorimetry
Remark: <= 5 mg/kg (ppm)
Test substance: Ammonium Sulfate Industrial Grade
Flag: non confidential, Critical study for SIDS endpoint
11-JUL-2003 (3)

EINECS-Name: organic carbon
Mol. Formula: C

Method: oxidation/infrared
Remark: typical value: 20-35 mg/kg (ppm)
Test substance: Ammonium Sulfate Industrial Grade
Flag: non confidential, Critical study for SIDS endpoint
11-JUL-2003 (3)

CAS-No: 7732-18-5
EC-No: 231-791-2
EINECS-Name: water
Mol. Formula: H2 O
Contents: <= .2 - % w/w

Method: analysis by weight
Test substance: Ammonium Sulfate Industrial Grade
Flag: non confidential, Critical study for SIDS endpoint
11-JUL-2003 (3)

EINECS-Name: free acid
Contents: <= .01 - % w/w

Method: titration
Test substance: Ammonium Sulfate Industrial Grade
Flag: non confidential, Critical study for SIDS endpoint
11-JUL-2003 (3)

1.4 Additives

1.5 Total Quantity

Remark: Production volumes for the year 2002:

Germany : approx. 0.76 Mill. t/a
Western Europe : approx. 3.95 Mill. t/a
USA/Canada : approx. 3.33 Mill. t/a
Asia (incl. Japan) : approx. 3.95 Mill. t/a

World : approx. 17.2 Mill. t/a
Flag: Critical study for SIDS endpoint
11-JUL-2003 (4)

1.6.1 Labelling

Labelling: no labelling required (no dangerous properties)

Flag: non confidential, Critical study for SIDS endpoint

26-MAY-2003 (1)

1.6.2 Classification

Classified: no classification required (no dangerous properties)

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (1)

1.6.3 Packaging

1.7 Use Pattern

Type: type
Category: Wide dispersive use

Flag: confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: industrial
Category: Agricultural industry

Flag: non confidential, Critical study for SIDS endpoint
04-FEB-1993

Type: industrial
Category: Chemical industry: used in synthesis

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: industrial
Category: Leather processing industry

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: industrial
Category: Metal extraction, refining and processing of metals

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: industrial
Category: Paper, pulp and board industry

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: industrial
Category: Textile processing industry

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: use
Category: Cleaning/washing agents and disinfectants

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: use
Category: Fertilizers

Flag: non confidential, Critical study for SIDS endpoint
04-FEB-1993

Type: use
Category: Flame retardants and fire preventing agents

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: use
Category: Food/foodstuff additives

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: use
Category: Laboratory chemicals

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: use
Category: other: fermentation processes

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: use
Category: other: nutrient for microorganisms in the production of enzymes

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: use
Category: other: soldering liquid

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: use
Category: other: use in electric dry cell batteries

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: use
Category: other: water treatment

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003

Type: use

Remark: Ammonium sulfate is used predominantly as a fertilizer. As a fertilizer, (NH4)2SO4 has the advantage of adding sulfur to the soil as well as nitrogen, as it contains 21 % by weight nitrogen and 24 % by weight sulfur.
Ammonium sulfate is also used in electric dry cell batteries, as a soldering liquid, as a fire retardant for fabrics and other products and as a source of certain ammonium chemicals. Other uses include water treatment, fermentation processes, fireproofing agents and leather tanning.
Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (5)

Type: use

Remark: batteries-, photo- and glass-industry
Flag: non confidential, Critical study for SIDS endpoint
11-MAY-2004 (6)

1.7.1 Detailed Use Pattern

1.7.2 Methods of Manufacture

Orig. of Subst.: Synthesis
Type: Production

Remark: Large-scale industrial processes (of caprolactam) are, without exception, multistage processes in which ammonium sulfate and sometimes organic compounds are formed as byproducts.
Flag: non confidential, Critical study for SIDS endpoint

04-APR-2002 (6)

Orig. of Subst.: Synthesis
Type: Production

Remark: Ammonium sulfate was originally manufactured by using sulphuric acid to scrub by-product ammonia from coke-oven gas, and much is still produced in this manner.
Most of the ammonium sulfate produced is now made by reaction between synthetic ammonia and sulfuric acid.

2NH3 + H2SO4 -> (NH4)2SO4

Where sulfur for sulfuric acid is at a premium, a process based on gypsum and carbon dioxide from combustion can be used:

(NH4)2CO3 + CaSO4*2H2O -> CaCO3 + 2H2O + (NH4)2SO4

Water is removed by evaporation, and the product is crystallized to large, white uniform crystals.
Anhydride (CaSO4) can also be used in this process.

Large quantities of ammonium sulfate are produced by a variety of industrial neutralization operations required for alleviation of stream pollution by free sulfuric acid (H2SO4) as well as in the manufacture of caprolactam.

Ammonium sulfate also is a byproduct of coke oven operations where the excess ammonia formed is neutralized with sulfuric acid to form the ammonium sulfate.

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (5)

Orig. of Subst.: Synthesis
Type: Production

Remark: In the laboratory by neutralization of diluted sulfuric acid with hydrous ammonia and careful evaporation.

Technical by discharging NH3 in sulfuric acid (80 %) or by discharging NH3 and CO2 in a suspension of CaSO4, whereby ammonium sulfate is seperated as solution.

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (2)

1.8 Regulatory Measures

1.8.1 Occupational Exposure Limit Values

Type of limit: MAK (DE)
Limit value: other: no MAK value established

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (7)

1.8.2 Acceptable Residues Levels

1.8.3 Water Pollution

Classified by: other: VwVwS (Germany), Annex 2
Labelled by: other: VwVwS (Germany), Annex 2
Class of danger: 1 (weakly water polluting)

Country: Germany
Remark: ID-Number: 296
Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (8)

1.8.4 Major Accident Hazards

1.8.5 Air Pollution

1.8.6 Listings e.g. Chemical Inventories

Type: EINECS
Additional Info: EINECS No. 231-984-1

Flag: non confidential, Critical study for SIDS endpoint
04-APR-2002 (9)

Type: ENCS
Additional Info: ENCS No. 1-400

Remark: ENCS Classification:
Inorganic Compounds
Flag: non confidential, Critical study for SIDS endpoint
04-APR-2002 (9)

Type: ECL
Additional Info: ECL Serial No. KE-01743

Flag: non confidential, Critical study for SIDS endpoint
04-APR-2002 (9)

Type: other: SWISS
Additional Info: SWISS No. G-1121

Remark: SWISS classification:
Giftliste 1 (List of Toxic Substances 1), 31 May 1999.
Toxic Category 5: Acute oral lethal dose of 2000-5000 mg/kg.
Flag: non confidential, Critical study for SIDS endpoint
04-APR-2002 (9)

Type: TSCA

Flag: non confidential, Critical study for SIDS endpoint
04-APR-2002 (9)

Type: DSL

Flag: non confidential, Critical study for SIDS endpoint
04-APR-2002 (9)

Type: PICCS

Flag: non confidential, Critical study for SIDS endpoint
04-APR-2002 (9)

Type: AICS

Flag: non confidential, Critical study for SIDS endpoint
04-APR-2002 (9)

Type: other: EU. Commission Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. O.J. (L 132) 1, 1 Jun 1996
Additional Info: From Part I: Cosmetic Ingredients other than Perfume and Aromatic Raw Materials.

24-NOV-2003

1.9.1 Degradation/Transformation Products

Type: thermal breakdown products
CAS-No: 7664-41-7
EC-No: 231-635-3
EINECS-Name: ammonia, anhydrous

Remark: at > 235°C
Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (1)

Type: reaction product
CAS-No: 7664-41-7
EC-No: 231-635-3
EINECS-Name: ammonia, anhydrous

Remark: Evolution of ammonia under influence of alkalies.
Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (1)

Type: thermal breakdown products
CAS-No: 7664-41-7
EC-No: 231-635-3
EINECS-Name: ammonia, anhydrous

Remark: When heating solid ammonium sulphate above 100°C, ammonium hydrogensulphate ((NH4)HSO4) is formed under release of ammonia (NH3).
Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (2)

Type: reaction product
CAS-No: 7803-63-6
EC-No: 232-265-5
EINECS-Name: ammonium hydrogensulphate

Remark: When heating solid ammonium sulphate above 100°C, ammonium hydrogensulphate ((NH4)HSO4) is formed under release of ammonia (NH3).
Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (2)

1.9.2 Components

1.10 Source of Exposure

Exposure to the: Substance

Remark: Ammonium sulphate is found naturally in volcanic craters.
Flag: non confidential, Critical study for SIDS endpoint
26-JAN-2004 (10)

Exposure to the: Substance

Remark: Ammonium sulphate may be formed as a by-product in the manufacture of caprolactam.

No more informations are available.
Flag: non confidential, Critical study for SIDS endpoint
26-JAN-2004 (10)

1.11 Additional Remarks

Memo: Reacts with alkalies and nitrites.

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (1)

1.12 Last Literature Search

1.13 Reviews

Memo: WHO (1986), Ammonia

Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (11)

Memo: WHO (1996), Drinking water guideline

Flag: non confidential, Critical study for SIDS endpoint
13-APR-2006 (12)

2.1 Melting Point

Decomposition: yes at >= 150 - 280 degree C

Remark: On heating in an open system, the compound begins to decompose at about 150 °C, yielding ammonium bisulfate (NH4HSO4), and releasing NH3.
Complete decomposition at 336 to 357 °C.
Reliability: (2) valid with restrictions
data from standard reference book
Flag: Critical study for SIDS endpoint
26-JAN-2004 (13)

Decomposition: yes at > 280 degree C

Method: other: no data
GLP: no data

Reliability: (2) valid with restrictions
Data from standard reference handbook
Flag: Critical study for SIDS endpoint
26-JAN-2004 (14)

Value: ca. 350 degree C
Decomposition: yes at > 235 degree C

Reliability: (4) not assignable
manufacturer/producer data without proof
08-MAR-2003 (15)

Value: = 350 degree C

Method: other: calculated with MPBPWIN v1.40
Year: 2003

Reliability: (2) valid with restrictions
calculated with standard model
27-APR-2003 (16)

Value: ca. 513 degree C

Remark: closed system conditions with a minimum of free gas volume; heated at an extremely slow rate (< 0.2 deg C / min)
Reliability: (2) valid with restrictions
data from standard reference book
28-MAY-2004 (13)

2.2 Boiling Point

Value: > 150 degree C
Decomposition: yes

Remark: On heating in an open system, the compound begins to decompose at about 150 °C, yielding ammonium bisulfate

(NH4HSO4), and releasing NH3.
Complete decomposition at 336 to 357 °C.

Reliability: (2) valid with restrictions
data from standard reference book

Flag: Critical study for SIDS endpoint

28-MAY-2004 (13)

Value: = 467 degree C

Method: other: calculated from the entropy of formation of solid (NH4)2SO4

Test substance: other TS: 99.0% min Univar brand (NH4)2SO4 supplied by Ajax chemicals, and 99.5% min Analar brand (NH4)SO4 supplied by British Drug Houses.

Remark: 1) Boiling point of material of approximately 98.8% purity (maximum boiling mixture). Impurities are (NH4)HSO4 and H2SO4. Boiling point of pure material will be lower, as will boiling point of material with increased impurity content.
2) Literature reports of the boiling (or decomposition) temperature vary from approximately 200 to 400°C, depending upon the purity.

Reliability: (2) valid with restrictions
calculated from measured properties

27-APR-2003 (17)

2.3 Density

Type: density
Value: = 1.77 at 25 degree C

Method: other: not specified
GLP: no data

Reliability: (2) valid with restrictions
data from standard reference book

Flag: Critical study for SIDS endpoint

28-MAY-2004 (14)

Type: bulk density
Value: = 1.769 g/cm³ at 20 degree C

Method: other: not specified

Reliability: (4) not assignable
secondary citation

08-MAR-2003 (18)

Type: relative density
Value: = 1.7716 at 20 degree C

GLP: no
Test substance: other TS: ammonium sulfate, no further details

Reliability: (2) valid with restrictions
data from standard reference book

25-MAR-2003 (13)

Type: density
Value: = 1.766 g/cm³

Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
manufacturer/producer data without proof
25-MAR-2003 (15)

Type: bulk density
Value: = 1000 kg/m3

Test substance: as prescribed by 1.1 - 1.4

Reliability: (4) not assignable
manufacturer/producer data without proof
25-MAR-2003 (15)

2.3.1 Granulometry

Method: other: no data
GLP: no data
Test substance: other TS: ammonium sulfate, industrial grade

Result: typical particle size distribution for Industrial grade ammonium sulfate:
- > 2 um, max 4 %
- 1-2 um, min. 85%
- 0.5-1 um, max. 11%.

Reliability: (4) not assignable
manufacturer/producer data without proof

Flag: Critical study for SIDS endpoint
17-APR-2003 (19)

2.4 Vapour Pressure

Value: = .000000004053 hPa

Method: other (measured): ring oven technique
GLP: no
Test substance: other TS: 99.0% min (Univar) (NH4)2SO4 supplied by Ajax chemicals, and 99.5% min (Analar) (NH4)SO4 supplied by British Drug Houses.

Remark: equilibrium partial pressure of ammonia over solid (NH4)2SO4
The equilibrium partial pressure of ammonia over solid (NH4)2SO4 has been determined as a function of temperature and acid impurity content. At 25 °C a 99.99% pure (NH4)2SO4 sample has a NH3 partial pressure of 4*E-12 atmospheres, whereas a a 99% pure (NH4)2SO4 sample has a NH3 partial pressure of only 4*E-14 atm.
Reliability: (1) valid without restriction

study well documented
Flag: Critical study for SIDS endpoint
23-AUG-2005 (17)

Value: at 25 degree C

Method: other (calculated): MPBPWIN v1.40 (modified Grain Method)
Year: 2003

Result: 6.63E-22mmHg (=8.84E-20Pa)
Reliability: (2) valid with restrictions
calculated with standard program
26-JAN-2004 (16)

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: = -5.1 at 25 degree C

Method: OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year: 1988
GLP: no

Test substance: purity: 99%
Reliability: (4) not assignable
The OECD test method 107 applies only to pure, water soluble substances which do not dissociate or associate and which are not surface active. Therefore the validity of this method for ammonium sulfate is uncertain.

Flag: Critical study for SIDS endpoint
25-MAR-2003 (20)

Partition Coeff.: octanol-water
log Pow: = .48 at 25 degree C

Method: other (calculated): LOGKOW, v. 1.66

Reliability: (2) valid with restrictions
accepted calculation method
Flag: Critical study for SIDS endpoint
26-JAN-2004 (21)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 764 g/l at 20 degree C
Descr.: of very high solubility

Reliability: (2) valid with restrictions
data from standard reference book
Flag: Critical study for SIDS endpoint
30-JAN-2004 (14)

Solubility in: Water
Value: = 770 g/l at 25 degree C
Descr.: of very high solubility

Reliability: (2) valid with restrictions
data from standard reference book
27-JAN-2004 (22)

Solubility in: Water
pH value: = 5 - 6

Remark: atmospheric aerosols; pH varies with relative humidity.
Reliability: (2) valid with restrictions
limited documentation
Flag: Critical study for SIDS endpoint
27-JAN-2004 (23)

Solubility in: Water
Value: = 754 g/l at 20 degree C
pH value: ca. 5
Conc.: 100 g/l degree C

Reliability: (4) not assignable
manufacturer data without proof
Flag: Critical study for SIDS endpoint
11-MAY-2004 (15)

Solubility in: Water
Value: = 843 g/l at 50 degree C
pH value: ca. 5
Conc.: 100 g/l degree C

Reliability: (4) not assignable
manufacturer/producer data without proof
22-APR-2003 (15)

Solubility in: Water
Value: ca. 430 g/l at 20 degree C

Reliability: (2) valid with restrictions
data from standard reference handbook
11-MAY-2004 (13)

Solubility in: Water
pH value: = 5

Method: other: not specified

Reliability: (4) not assignable
secondary citation
21-MAY-2003 (18)

Solubility in: Water

Value: = 1040 g/l at 100 degree C
Descr.: of very high solubility

Reliability: (2) valid with restrictions
data from standard reference book
22-APR-2003 (22)

Solubility in: Water
pH value: ca. 5
Conc.: 100 g/l degree C

Reliability: (4) not assignable
manufacturer/producer data without proof
17-APR-2003

2.6.2 Surface Tension

2.7 Flash Point

2.8 Auto Flammability

2.9 Flammability

Result: non flammable

Method: other: no data
GLP: no data

Reliability: (4) not assignable
secondary citation
17-APR-2003 (24)

2.10 Explosive Properties

Result: May explode if mixed with oxidizers, such as potassium nitrate, potassium nitrite and potassium chlorate.
Reliability: (4) not assignable
Secondary quotation
27-JAN-2004 (24)

2.11 Oxidizing Properties

Result: no oxidizing properties

Reliability: (4) not assignable
secondary citation
08-MAR-2003 (24)

2.12 Dissociation Constant

2.13 Viscosity

2.14 Additional Remarks

Remark: Thermal decomposition products: ammonia.
Formation of ammonia in contact with alkaline solutions.
Dangerous reactions with: nitrites.
Reliability: (4) not assignable
manufacturer/producer data without proof
26-MAY-2000 (25)

3.1.1 Photodegradation

Remark: Ammonium sulfate is not photodegraded. It can be formed in atmospheric aerosols from the interaction of atmospheric ammonia with atmospheric SO2.

22-MAY-2003 (26) (17)

3.1.2 Stability in Water

Type: abiotic

Remark: In aqueous solution, ammonium sulfate is completely dissociated into the ammonium ion (NH4+) and the sulfate anion (SO42-). Depending on pH, ammonia (NH3) exists in equilibrium with the ammonium ion (NH4+), according to the following relationship:

NH4+ + H2O <-> NH3 + H3O+

In general, as pH increases, the fraction of the total ammonia which is un-ionized increases. For example, at 5 °C and pH 6.5, 0.0395% of the total ammonia is present as NH3. Increasing the pH from 6.5 to 8.5 will increase the un-ionized ammonium by a factor of approximately 100 (Rice and Bailey 1980). Increasing the temperature will also increase the percentage of unionized ammonium. For example, in seawater at 25 °C and pH of approximately 8.1, approximately 7% of the total ammonia is present as NH3.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

12-JUL-2004 (27) (28)

3.1.3 Stability in Soil

3.2.1 Monitoring Data (Environment)

Type of measurement: background concentration
Medium: air

Method: Fine (<2.5µm) and coarse (2.5-152.5µm) particles were collected by samplers located within an assembly factory, in equipment rooms and on the roofs of these buildings. The sampling interval was 84 hours, and the sampling was conducted for 9 months, from June 1988. The equipment rooms were located at Newark NJ and Neenah WI, while the factory was located in the northeastern part of the USA.

Result: Average outdoor total fine-particle, ammonium, and sulfate concentrations ranged from 13600 - 18090 ng/m3, 1573-1840 ng/m3, and 3800-5214 ng/m3, respectively.
Indoor concentrations were greatest in the manufacturing plant, with average total fine-particle, ammonium, and sulfate concentrations being 12200 ng/m3, 846 ng/m3, and 2840 ng/m3, respectively. The electronic equipment rooms had lower levels of fine particles (1600 and 2880 ng m-3), ammonium (190 and 168 ng/m3), and sulfate (620 and 721 ng/m3).

Average outdoor total coarse-particle, ammonium, and sulfate concentrations ranged from 6590 - 12340 ng/m3 , 1-26 ng/m3 and 230 - 725 ng/m3, respectively.
Indoor concentrations of ammonium and sulfate were <13 and <104 ng/m3, respectively.

Test substance: ammonium and sulfate, obtained from sampled air
Reliability: (1) valid without restriction
study well documented and assignable
Flag: Critical study for SIDS endpoint
28-MAY-2004 (29)

Type of measurement: background concentration
Medium: air

Method: 24 hr air particle sampling was conducted at 20 residences in Connecticut between August 1994 and June 1995, and at 261 homes (nonsmoker) in southwest and central Virginia between July 1995 and January 1998. 58 residences were sampled in the summer, and 223 during the winter. Central site ambient air sampling was conducted at Vinton, VA - a site selected to represent regional air quality.

Result: The mean summertime concentration of ammonium at regional sites was 124.6 +/- 59.0 nmol/m3 (daily range 30.6-293 nmol/m3). The mean summertime concentration of ammonium outside of homes was 129.4 +/- 87.8 nmol/m3 (daily range 0-338.9 nmol/m3).
Inside air conditioned homes (n = 49), the mean summertime concentration of ammonium was 78.3 +/- 77.2 nmol/m3 (daily range 0-450.6 nmol/m3).
Inside non-air conditioned homes (n=9), the mean summertime concentration of ammonium was 96.7 +/- 68.9 nmol/m3 (daily range 6.7 - 214.4 nmol/m3).
Averaged summer sulfate air concentrations were 88.4 +/- 51.6 nmol/m3 (daily range 14.1 -209 nmol/m3) at the regional center, and 83.7+/- 53.7 nmol/m3 (daily range 7.9-230.6 nmol/m3) outside the homes. Inside air conditioned and non air conditioned
homes, the averaged air concentrations of sulfate were 47.8+/- 36.3 nmol/m3 (daily range 2.1-137.7 nmol/m3) and 63.0 +/- 37.3 nmol/m3 (daily range 20.9 - 125.6 nmol/m3, respectively. In winter, the averaged air concentration of ammonium outside of homes was 64.4+/- 38.9nmol/m3 (daily range 2.2-187.8nmol/m3). Homes without kerosene heaters had an averaged air concentration of ammonium of 9.4+/- 21.7 nmol/m3 (daily range 0 -125.0 nmol/m3). Homes with kerosene heaters had an averaged air concentration of ammonium of 126.1 +/- 155.0 nmol/m3 (daily range 0.6 -796.7 nmol/m3).
Winter sulfate concentrations outside of homes averaged 30.6+/- 14.9 nmol/m3 (daily range 0-66.2 nmol/m3). Homes without kerosene heaters had an averaged air concentration of sulfate of 21.6+/- 3.37 nmol/m3 (daily range 0 -146.5 nmol/m3). Homes with kerosene heaters had an averaged air concentration of sulfate of 82.7 +/- 76.2 nmol/m3 (daily range 6.1 -379.6 nmol/m3.)

Test substance: ammonium and sulfate, obtained from sampled air
Reliability: (1) valid without restriction
study well documented and assignable
Flag: Critical study for SIDS endpoint
28-MAY-2004 (30)

Type of measurement: background concentration
Medium: sediment

Remark: Ammonium sulfate is found naturally in volcanic craters.
Flag: Critical study for SIDS endpoint
26-JAN-2004 (10)

Type of measurement: background concentration

Remark: In the frame of the German water quality monitoring program ammonium nitrogen concentrations were measured. In 2000, the rivers Danube, Oder, Weser, Rhine, and Elbe showed concentrations (50th percentile) ranging from 0.04 to 0.07 mg/l.
UBA states that in Germany 624 000 t ammonia were emitted to air in 1999
23-AUG-2005 (31)

Type of measurement: concentration at contaminated site
Medium: other: rainwater

Method: Monthly bulk precipitation samples were collected on a rural coastal island located 8-10 km from the NL mainland, for more than 15 years (May 1972 - December 1987). Comparative samples for an urban environment were obtained from a region southeast of Amsterdam, for the years 1973-1982 inclusive.
Remark: Monthly bulk precipitation
Result: Higher levels of ammonium and sulfate in bulked rainwater were found in the coastal island rural environment than at Ouderkerk. The higher rural concentration of ammonium at Schiermonnikoog is ascribed to dry deposited NH3 in the collectors, due to substantial cattle breeding.
The precipitation weighted averages of monthly concentreations of the dissolved substances in bulk precipitation for Schiermonnkoog during May 1972 - 1987 were 2.684 mg/l (converted from 149.1 µmol/l) and 6.65 mg/l (converted from 69.3 µmol/l) respectively, with seasonal variations showing higher values in late winter/early spring, and lower values in autumn. For Ouderkerk no concentrations were reported.
Test substance: ammonium and sulfate, obtained from rainwater.
Reliability: (1) valid without restriction
study well documented and assignable
Flag: Critical study for SIDS endpoint
28-MAY-2004 (32)

Type of measurement: other: calculation of consumer exposure

Remark: Sulfates are natural components of food; ammonium sulfate is "generally recognized as safe (GRAS)" and approved as a food additive in the U.S. (FDA 2003) and in Europe (E 517; EU, 1995). From this data it can be seen that consumer exposure to sulfate is low: 453 mg per day via food, 48 mg per day via drinking water assuming 2 l drinking water per day and 0.63 ug per day via air assuming 20 m3 respiratory volume. Ammonia intake via food is 18 mg/day. Endogenous production of ammonia (4000 mg/day) is about 2 orders of magnitude higher than exogenous intake (ammonia and ammonium) via food (20 mg/day), air (< 1 mg/day) and water (< 1mg/day) (WHO, 1986, 1996,

2003).
Reliability: (2) valid with restrictions
18-APR-2006 (33) (34) (11) (12) (35)

Type of measurement: other
Medium: food

Remark: Small amounts of ammonium sulfate are used in food/food stuff additives. Average amounts used were reported as 0.033% in baked goods, and as 0.075% in gelatins, puddings, and custard.
Reliability: (4) not assignable
secondary citation
21-APR-2003 (36)

3.2.2 Field Studies

Type of measurement: other: Leaching from soil- laboratory or greenhouse experiments
Media: Leachate from lysimeter

Method: Artificial soil profiles were constructed in PVC cylinders (155 mm inner diameter), with bottom plates and 15 µm pore size suction filters connected to an Erlenmeyer flask for leachate removal. The parent soil was a glacial till with sand and fine sand dominating, which had received 110 kg N ha-1 yr--1 ammonium nitrate as fertiliser for 20 years.
The profiles consisted of 50 mm O (O: humus; 43%C, 1.3%N, pH H2O 4.1) and 100 mm E (2.9% C, 0.10%N, pH H2O 4.4) horizons (A columns), or 50 mm O (43% C, 1.3%N, pH H2O 4.1), 100 mm E (2.9% C, 0.10%N, pH H2O 4.4) and 100 mm B (2.5% C, 0.10%N, pH H2O 4.9) horizons (B columns).
4 replicates were run for each experimental situation, which comprised bare and vegetated soil (grass, and ammonium sulfate at 0.0025 (control), 0.25,and 0.5 mM/l and 2 profile depths.
Irrigation was performed manually at irregular intervals, generally about once a week, as intense rain showers of 5.3 mm. Just before sampling the irrigation rate was increased to 5.3 mm per day, to make leachate collection possible.
Remark: These laboratory or greenhouse experiments with repacked soil all demonstrate that soil texture, clay content, and pH are important in controlling NH4+ leaching, with more leaching occurring with sandy soils at a low pH. The ionic strength of the irrigation water may also be important, as may be the level of application of ammonium sulfate. More leaching is observed from bare soil than in the presence of crops.
Result: In the absence of vegetation up to 80 % of applied NH4+ was recovered in the leachate in the A columns (O and E horizons only) for the higher level (0.5mM) ammonium sulfate solution.
In the presence of vegetation, up to 50% of applied NH4+ was recovered in the leachate. At the lower level (0.25 mmM) of application very little NH4+ was
recovered in the leachate, in both the presence and the absence of vegetation. The B columns, which contained soil from the B horizon as well as from the O and E horizons, showed reduced NH4+ in the leachate, with only about 30% of NH4+ being found in leachate for the 0.5 mM application in

both bare and vegetated soil. Negligible NH4+ was detected in the leachate from the 0.25 mM application.
Test substance: ammonium sulfate (NH4)2SO4, not specified further.
Reliability: (1) valid without restriction
study well documented and assignable
04-JUN-2004 (37)

Type of measurement: other: Leaching from soil- laboratory or greenhouse experiments
Media: Leachate from lysimeter

Method: soil (a sandy over loamy, mixed, mesic Ustic Haplocalcid) was collected from the top 30 cm of an uncultivated site typical of agricultural areas near Green River, UT. The soil was air dried, sieved to <2mm, and 6.5 kg soil was placed in each 6 l pot. Each ammonium sulfate amendment, at 112 or 224 kg N ha-1, was located off the centre, and in strips about 2.5 cm wide and 11 cm long. Each treatment was replicated 3 times. After wetting the soil in each pot with 1500 ml irrigation water, 500 ml of irrigation water (pH 8.12, EC 0.675 dS m-1) was added to each pot every 4th day of the 40 day experiment. Temperatures in the greenhouse were ca. 16 °C during the night and ca. 30 °C during the day. River water was chosen for irrigation, as distilled water did not leach ammonium from the soil. Leachate was collected, immediately frozen, and later analysed for NH4+ N. Total leachate collected during the experiment was approximately 1 pore volume. A similar experiment was carried out with acid washed rounded quartz
sand.
Remark: These laboratory or greenhouse experiments with repacked soil all demonstrate that soil texture, clay content, and pH are important in controlling NH4+ leaching, with more leaching occurring with sandy soils at a low pH. The ionic strength of the irrigation water may also be important, as may be the level of application of ammonium sulfate. More leaching is observed from bare soil than in the presence of crops.
Result: By day 40, 3% of the 112 kg N ha-1 and 18% of the 224 kg N ha-1 leached as NH4+ -N from the ammonium sulfate amended soil. With sand as the substrate, more than 91% of the ammonium applied had leached from the system after 4 days, regardless of the application rate.
Test substance: ammonium sulfate (NH4)2SO4, not specified further.
Reliability: (1) valid without restriction
study well documented and assignable
28-MAY-2004 (38)

Type of measurement: other: Leaching from soil- laboratory or greenhouse experiments
Media: Leachate from lysimeter

Method: Air dried Myakka fine sand (sandy, silicious hyperthermic Aeric Haplaquods) was packed in 16cm inner diameter, 67 cm length PVC columns. The soil horizons were packed to closely resemble the soil profile in the field. The depth of the horizons were from 0-20 cm for Ap (97.4 % sand, 1.9% silt, 0.8% clay, pH 4.9, 8% organic matter), 20-46 cm for A22 (98.5 % sand, 1.2% silt, 0.3% clay, pH 5.9, 1% organic

matter), and 46-66 cm for Bh (94.2 % sand, 2.5% silt, 3.3% clay, pH 4.6, 29.8% organic matter). Bahiagrass sod was planted on each column, and fertilizers were applied 2 weeks later. Distilled water was applied to each soil column prior to fertilization, and the colunms were allowed to drain to field capacity. Ammonium sulfate was applied to three replicates of soil columns at 0, 84, and 168 kg N ha-1 (randomised block design). In addition, all columns received 45 kg P ha-1 as triple phosphate, and 90 kg K ha-1 as KCl. 29.2 mm Irrigation water (content not specified - may be distilled water?) was applied every 4th day for 3 months. The effluent was collected from the bottom of each column for 24 hours after each irrigation treatment. A subsample of the leachate was frozen, and later analysed for NH4+ N.

Remark: These laboratory or greenhouse experiments with repacked soil all demonstrate that soil texture, clay content, and pH are important in controlling NH4+ leaching, with more leaching occurring with sandy soils at a low pH. The ionic strength of the irrigation water may also be important, as may be the level of application of ammonium sulfate. More leaching is observed from bare soil than in the presence of crops.

Result: For all fertiliser treatments the amount of NH4+ N leached was less than 1%.

Test substance: ammonium sulfate (NH4)2SO4, not specified further.

Reliability: (2) valid with restrictions
no data on the composition of the irrigation water.

18-MAY-2004 (39)

Type of measurement: other
Media: stream water

Method: The study was conducted to determine the response of stream water DOC and organic acidity to increased inputs of ammonium sulfate to a whole catchment. Precipitation, throughfall, and soil solution (from Spodosols) and stream waters were characterized for DOC concentrations and fractions (hydrophobic acids and neutrals, hydrophilic acids, bases, and neutrals) in both a control ("East Bear" Brook) and the treatment ("West Bear" Brook) catchments of Bear Brook Watershed, Maine / USA, a northern hardwood forest.

Result: There were no clear, detectable changes in stream water dissolved organic carbon (DOC), with only minor changes in organic anions, as a result of bimonthly treatment with ammonium sulfate at a dose of 900 mol/ha/year since November 1989. The treatment has resulted in an increase in N inputs from ambient values of 600 mol/ha/year to 2400 mol N/ha/year and an increase in ambient S input of 450 mol/ha/year to 1350 mol/ha/year.

Reliability: (1) valid without restriction
study well documented and assignable

28-MAY-2004 (40)

Type of measurement: other: leaching from soil
Media: stream water

Method: A helicopter was used to apply bimonthly applications of

pelletized 15N labelled ammonium sulfate fertiliser to the West Bear Brook catchment, from June 1991 until December 1992 (1991: 21 kg N/ha, 1992: 42 kg/ha). This was part of an experiment involving otherwise unlabelled ammonium sulfate fertiliser application at the same level, which has run from 1989 to 1996. The nearby East Bear Brook catchment, with no fertiliser application, was used as a control.

Remark: Further information on soil characterisation may be available in Norton et al (1999), in Environmental Monitoring and Assessment 55 (page not given).

Result: Analysis of stream water established that the 1.5 year (1991-1993) cumulative exports of fertiliser-derived ammonium represented less than 1% of the labelled fertiliser added to the catchment. Nitrate plus ammonium exports contained ca. 2 kg N/ha of the 42 N kg/ha deposited.

Test substance: Commercial pelletised ammonium sulfate fertiliser, enriched in 15N at the Tenessee Valley Authority laboratory by dissolution of the pellets in water, addition of (15NH4)2SO4 into the fertiliser solution, and repelletizing the precipitated, labelled fertiliser.

Reliability: (1) valid without restriction
study well documented and assignable

28-MAY-2004 (41)

Type of measurement: other
Media: soil

Result: In two soils treated with ammonium sulfate at rates of 0, 25, 50, 100 and 150 mg N/kg ammonium was rapidly converted to nitrate. In Tifton soil (fine loamy, siliceous, thermic Plinthic Paleudults) practically all ammonium had disappeared by day 22, whilst nitrification in Dothan soil, a fine loamy, siliceous, thermic Typic Hapludults, was much slower, and almost stopped by day 30. This difference between soils was probably due to differences in soil pH and buffer capacity. Tifton soil had an initial pH of 6.8, wihich is considered to be close to the optimum for nitrification. In contrast, Dothan soil had an initial pH of 5.5, providing a much less favorable environment for nitrification. Nitrification in Dothan soil stopped when thepH approximated a value of 4.5.

Test condition: temperature: 30 °C
test duration: 40 d in quadruplicate
air change: each 2. day

Reliability: (1) valid without restriction
study well documented and assignable

28-MAY-2004 (42)

Type of measurement: other: laboratory study
Media: soil-air

Remark: 1) This is a static test, so less ammonia will be removed than in a flow through test.
2) This reference also cites other papers which give ammonia losses from fertilisers and liquid sludges applied to soils of up to 60%. The ammonia volatilisation is dependent upon the pH of the soil and mode of application, with subsurface application having greatly reduced ammonia loss from soil.

Surface crop residue may also be important in some cases.
Result: Only 1% or less of the applied ammonium sulfate was captured as ammonia, after 16 days at 25 °C.
Test condition: In a laboratory study using sieved soils hydrated to field capacity, static ammonia traps were used to capture ammonia volatilising from ammonium sulfate added to two soils of pH 6.8 and 5.6, with and without a straw cover.
Reliability: (3) invalid
incomplete documentation

18-MAY-2004 (43)

Type of measurement: other
Media: soil-air

Result: 40% of the total applied ammoniacal nitrogen was volatilised after 48 hours.
Nitrification did not occur in the soil, due to the high concentration of applied N (about 870 µg NH4-N/g soil).
Test condition: 15N-labelled ammonium sulfate was added to a pig slurry, and labelled 15-N ammonia loss from soil to which pig slurry was topically applied was measured in gas volatilisation chambers. The air flow rate was 5 L/min (about 10-12 exchange volumes per minute).
At each sample time the soil/slurry mixture was extracted. Extract and extract residue were analyzed for total ammonical nitrogen, NO3-N and total N-content. NH3 volatilizing from the soil/slurry mixture was measured. Three replicates for each treatment were conducted
Reliability: (2) valid with restrictions
basic data given

28-MAY-2004 (44)

Type of measurement: other
Media: soil, biota, precipitation, streamwater

Method: A 15N-tracer study in a fertilized, forested catchment at the Bear Brook Watershed in Maine, USA, was conducted in order to characterize N cycling processes, to identify sinks for ammonium-N additions, and to determine the contribution of the experimental ammonium additions to nitrate export from the treated catchment.
Result: Distributions of 15N in plant tissues, soils, precipitation and streamwater was collected before adding tracers and showed that nitrate-N (the dominant form of inorganic N deposition at the site) inputs under ambient conditions were depleted in 15N relative to plants and that soil was enriched in 15N relative to plants. The 15N content of streamwater nitrate was within the range of 15N contents in natural plant tissues, suggesting that nitrate deposited from the atmosphere is reduced and assimilated into soil and plant N pools before being leached as nitrate from the catchment. Variations in 15N natural abundances also suggested that most N uptake by trees is from the forest floor and that nitrification occurs in soils at this catchment under ambient conditions. Changes in 15N contents of plant tissues, soils and streamwater after adding a 15N tracer to the ammonium sulfate fertilizer applied to the

treated catchment showed that soils were the dominant sink for the labeled ammoniun. Surface soils (Oea horizon plus any underlying mineral soil to 5 cm depth) assimilated 19 to 31 percent of the 42 kg/ha of labeled ammonium-N additions. Of the three forest types on the catchment, the soil: biomass assimilation ratio of labeled-N was highest in the spruce forest, intermediate in the beech-dominated hardwood forest and lowest in the mixed hardwood-spruce forest. Although ammonium sulfate additions led to increases in streamwater nitrate, only 2 of the 13 kg/ha of nitrate-N exported from the catchment during the 2 years of tracer additions was derived from the 42 kg/ha of labeled ammonium-N addition.

Reliability: (1) valid without restriction
study well documented and assignable

28-MAY-2004 (41)

Media: soil-biota-air

Method: Various N fertilizers were applied to winter wheat under field conditions. Ammonium sulfate was distributed at the beginning of tillering, and was tested at 16 g N/m2 in a microplot experiment. Gas traps of 4.4 dm3 were laid between the rows of the microplots and ammonia, nitric oxide, nitrous oxide, dinitrogen oxide and nitrogen contents of trapped soil air were analyzed by gas chromatography duringn vegetation.

Result: The N losses measured at the different samplings were not higher than 1.2% of the N doses applied in the field. The complete transformation of the fertilizers took place practically within 11 weeks after their distribution to wheat grown in the field. The composition of the nitrogenous gases changed during vegetation depending on the applied fertilizer. The proportion of N2O increased within the total amount of nitrogenous gases in the field traps. Among the nitrogenous gases tested, N2 showed by far the highest and NO the lowest concentration.

Test condition: slightly acidic brown forest soil
measurement period: 13, 41, 62, 76 and 108 d

Reliability: (1) valid without restriction
study well documented and assignable

28-MAY-2004 (45)

Type of measurement: other: leaching from soil
Media: leachate from lysimeter

Method: An identical experimental protocol was followed in six European coniferous forest soils, with different soil characteristics, pollution histories and climate, to measure the effects of enhanced (NH4)2SO4 deposition on lysimeter throughfall. The sites are located in: Clonegal (IRL) - brown soil, clay/silty clay, stand = Picea abies, cation exchange buffer range, C/N 14.5, past N input 9.6 kg/ha/a, pHH2O 4.2.Fontainebleau (FR), - Podsol soil, loamy sand, stand = Pinus sylvestris, aluminium buffer range, C/N 27.0, past N input 10.5 kg/ha/a, pHH2O 3.9.Grizedale (UK) - brown soil, clay, stand = Picea abies, aluminium buffer range, C/N 15.8, past N input 13.3 kg/ha/a, pHH2O 3.7.Haldon (UK) -brown soil, clay loam, stand = Picea abies, cation exchange buffer range, C/N 13.8, past N input 17.3 kg/ha/a, pHH2 4.3.Solling (DE) -

acid brown soil, silty clay loam, stand = Picea abies, aluminium buffer range, C/N 17.0, past N input 37.1 kg/ha/a, pHH2O 3.3.and Wekerom (NL) - Podsol soil, sand, stand = Pinus sylvestris, aluminium buffer range, C 22.0, past N input 52.1 kg/ha/a, pHH2O 3.9.Cation exchange capacity and base saturation information can be found in the paper.
Lysimeters were prepared from soil cores (14 cm inner diameter and 24 cm deep) taken at each site in Plexiglas cylinders. The bases of the cores were trimmed, and a 5 cm deep layer of acid washed sand was placed at the bottom of each core, which was then capped and sealed.
A ceramic cup conected to a suction apparatus was inserted diagonally into the sand layer, and the join with the cykinder was sealed.
The throughfall solution was removed through the ceramic cup during the experiment. 28 lysimeters were installed at each site, using a randomised block design. The lysimeters were roofed to enable control of throughfall inputs. One half of the lysimeters contained carefully inserted living tree roots. Throughfall applications consisting of the throughfall volumes of each individual site, collected and measured for the period, with the addition of 75 kg ha-1-a-1 (NH4)2SO4 for the N treatments, were made to each lysimeter at 2 week intervals. The leachates were collected every 2 weeks, for 1 year from September 1992, and analysed for volume, pH, and major cations and anions.

Result: Soil texture and pH were important in controlling NH4+ leaching. The two less acidic, clay/clay loam soils showed almost total retention of NH4+. Nearly 75% of added N was leached as NH4+ in the acid sandy soils. The presence of living roots significantly reduced soil solution nitrate and associated cation concentrations at two of the six sites. The very different responses of the six soils to increased (NH4)2SO4 deposition emphasises that the establishment of N critical loads for forest soils needs to allow for differences in the N storage capacity and nitrification potential of the soils

Test substance: ammonium sulfate (NH4)2SO4, not specified further

Reliability: (2) valid with restrictions
study well documented and assignable

Flag: Critical study for SIDS endpoint

04-JUN-2004 (46) (47)

3.3.1 Transport between Environmental Compartments

Type: adsorption

Media: water - sediment

Result: log Koc = 1.38 (Koc = 24)

Reliability: (2) valid with restrictions
accepted calculation method

Flag: Critical study for SIDS endpoint

27-JAN-2004 (16)

Media: soil - air

Method: other: measured

Remark: 1) This is a static test, so less ammonia will be removed

than in a flow through test.
2) This reference also cites other papers which give ammonia losses from fertilisers and liquid sludges applied to soils of up to 60%. The ammonia volatilisation is dependent upon the pH of the soil and mode of application, with subsurface application having greatly reduced ammonia loss from soil. Surface crop residue may also be important in some cases.

Result: Only 1% or less of the applied ammonium sulfate was captured as ammonia, after 16 days at 25 °C.

Test condition: In a laboratory study using seived soils hydrated to field capacity, static ammonia traps were used to capture ammonia volatilising from ammonium sulfate added to two soils of pH 6.8 and 5.6, with and without a straw cover.

Reliability: (3) invalid
incomplete documentation

Flag: Critical study for SIDS endpoint

18-MAY-2004 (43)

3.3.2 Distribution

Media: air - biota - sediment(s) - soil - water

Method: other (calculation)

Remark: Fugacity model cannot be used due to inorganic nature of the substance. Based on the physical-chemical properties of the substance water is probably the preferred compartment.

15-JUL-2004

3.4 Mode of Degradation in Actual Use

3.5 Biodegradation

Method: other

Remark: Due to the inorganic nature of the substance standard testing systems are not applicable.

12-JUL-2004

3.6 BOD5, COD or BOD5/COD Ratio

3.7 Bioaccumulation

Remark: Based on a log Kow of - 5.1 (measured), and 0.48 (calculated), bioaccumulation is not expected.

Reliability: (2) valid with restrictions

23-AUG-2005

3.8 Additional Remarks

Memo: other

Result: The U.S. Environmental Protection Agency has analyzed data tabulated from the Agency`s STORET data base for all 50 states and found that at the 50th percentile for pH and temperature in surface waters, approximately 1 percent of aqueous ammonia would exist in the un-ionized form, at the 90th percentile it would be 10 percent, and at the 95th percentile it would be 15 percent.

Reliability: (4) not assignable
Secondary quotation

18-MAY-2004 (48)

Memo: other

Result: Aqueous ammonia does not persist or bioaccumulate in the environment as ammonia. In surface waters the important and competitive processes that remove aqueous ammonia are nitrification and volatilisation. The rate of volatilization of ammonia from surface waters is highest at the sources of releases, while nitrification processes tend to be more significant in lakes, slow moving rivers, and estuaries. Nitrification, which is one process within the nitrogen cycle, involves two steps that yields metabolic energy for two specific microorganisms. In the first step, Nitrosomonas converts ammonia to nitrite and in the second step, Nitrobacter converts nitrite to nitrate. Because the nitrogen cycle is dynamic, industrial releases of aqueous ammonia should not result in dramatic buildups of ammonia in surface waters. Nitrification is responsive to high inputs of ammonia such as those from industrial releases. However, it should be noted that high nitrification may lead to low levels of dissolved oxygen and the eutrophication of a water body. This effect is typically limited to coastal waters and estuaries where nitrogen is the limiting nutrient. Aqueous ammonia may also be removed by adsorption to particles which then settle to the sediment where soil-type processes take over. The ionized form of ammonia is also assimilated by most plants.

Reliability: (4) not assignable
Secondary quotation

28-MAY-2004 (48)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC0: 464
LC50: 681
LC100: 1000
Limit Test: no

Method: other: equivalent to OECD 203
Year: 1989
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Result: Clinical signs included apathy and tumbling at 1000 mg/L. No signs of toxicity were noted at 100, 215, and 464 mg/L.

Mortalities at 96 hours:
100 mg/L: 0/10
215 mg/L: 0/10
464 mg/L: 0/10
1000 mg/L: 10/10

pH values after 1 hour were: 7.6, 7.5, 7.5, 7.3, 7.3 for control, 100, 215, 464, and 1000 mg/L, respectively.
pH values after 96 hours were: 7.8, 7.7, 7.7, 7.6, and not determined for control, 100, 215, 464, and 1000 mg/L, respectively.
Oxygen content (mg/L) after 1 hour was: 7.7, 8.3, 8.5, 8.5, 8.6 for control, 100, 215, 464, and 1000 mg/L, respectively.

Oxygen content (mg/L) after 96 hours was: 8.0, 8.1, 8.2, 8.0, and not determined for control, 100, 215, 464, and 1000 mg/L, respectively.

Test condition: TEST ORGANISMS: Golden orfe (Leuciscus idus L., golden variety), 2.9 g (2.1-3.6 g), 6.7 cm (6.1-7.2 cm). The test organisms were acclimated to the control diluent water in the breeding/rearing tanks for 3 days. Food was withheld for the 24 hours preceding start of the test and during the test. Ten fish were exposed to the test chemical in each treatment. Biological loading was 2.9 gram wet weight per liter of test solution.

TEST VESSEL: The tests were performed in all-glass aquaria of 30cmx22cmx24cm size to which 10 liters of test water were added.

TEST SOLUTION: The test chemical was added directly to the diluent water to give nominal concentrations of 100, 215, 464 and 1000 mg/L. The test chemical concentrations were not analyzed. Once the test solutions were prepared, the starting temperature, dissolved oxygen and pH values were determined for each exposure concentration.

When the starting pH of the test soluton fell outside the

extremes of 6.5 to 8.5, the pH was adjusted to 7.0 by the addition of 10% (v/v) NaOH or 10% (v/v) H2SO4.

DILUTION WATER: Water quality was routinely monitored to characterize the diluent water and ensure its suitability according to the standard method for the examination of water and wastewater DIN 38 412, part 11, October 1982. The chemical characteristics are reported in the publication and are in accordance with standards required by current testing guidelines.

PHYSICAL/CHEMICAL PARAMETERS:
Determination of the temperature, dissolved oxygen and pH of each test solution were made in conjunction with the daily biological observation. The test temperature target was 20 -21 °C. The photoperiod duration was 16 h of light.

BIOLOGICAL PARAMETERS: biological observations were made daily. Survival, condition and behavioral information were recorded. Dead organism were removed when observed.

EXPOSURE PERIOD: 96 hours.

STATISTICAL METHOD: The 96-h LC50 value was derived using the Probit method and geometric means.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
23-JAN-2004 (49)

Type: flow through
Species: Brachydanio rerio (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: = 480
Limit Test: no

Method: other: ISO/TC 147/SC 5/WG (1976)
GLP: no data
Test substance: other TS: ammonium sulfate, analytical grade

Result: The LC50 for NH4E+ was quoted to be 130 mg/l, 149 and 162 mg/l after 96, 48 and 24 hours, respectively.
The LC50 values for (NH4)2SO4 after 24 and 48 hours were 600 and 550 mg/L, respectively.
Test duration was with two ages:
1,8 g/5,9 cm and 2,1 g/6,2 cm.
The LC50 value based of the analytical N -determination:
LC50 related to NH3-N = 36.7 mg/l.
LC50 related to NH3 = 0.76 mg/l

The physico-chemical characteristics of the test water (temperature, pH and O2) showed only minor variations during the test period, as well as between different test aquaria in a test series (no details reported).

Test condition: TEST ORGANISMS: At least 14 days before the start of the experiment, the fishes were put in storage tanks in the experimental room. The fishes were fed once a day with commercial fish food. The light was automatically regulated

to give a 12 hours day period and 12 hours darkness.
TEST TEMPERATURE: 23.5 °C, thermostated experimental room.
DILUTION WATER: the dilution water was prepared accodring to the ISO proposal by dissolving the chemicals in deionized water. The water was prepared one day before the use and was kept in PVC tanks.
PILOT TEST: the test was preceded by a least one pilot test under static conditions. 5 fishes were exposed in each test jar, and six concentrations were tested.
CONTINUOS-FLOW TEST: the dilution water and the test substance were distributed by aid of peristaltic pumps through silicone rubber tubes to a mixing chamber (250 mL suction flask). the tubes ended at the bottom of the mixing chamber. Mixing was made by a magnetic stirrer. From the spout of the mixing chamber, the mixed solution was delivered to the test jars via silicone rubber tubes. The test jars consisted of 1 L spherical glass flasks with threen necks. The test water flow was approximately 1 L/hour during all the experiments. Six concentrations, including one control, were used in the tests. Ten fishes were exposed to each concentration. The condition of the fishes was checked after 3, 6, 12, 24, 48, 72 and 96 hours. At the same time, the dissoved oxygen content, pH and temperture were recorded.

Reliability: (2) valid with restrictions

limited documentation

Flag: Critical study for SIDS endpoint

23-JAN-2004 (50)

Type: semistatic
Species: Brachydanio rerio (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: = 420
Limit Test: no

Method: other: see Test Conditon
GLP: no data
Test substance: other TS: ammonium sulfate, analytical grade

Remark: The LC50 for NH4E+ was quoted to be 114, 130 and 141 mg/L after 96, 48 and 24 hours, respectively. The LC50 values for (NH4)2SO4 after 24 and 48 hours were 520 and 480 mg/L, respectively.

Test condition: Test Temperature: 23.5 °C, thermostated experimental room.

Test Jars: 10 L glass jars with 4 L of test substance. Every 24 hours the fishes were transferred, using a dip net, to jars with new test solutions. the test water was not aerated during the tests.

Reliability: (2) valid with restrictions
limited documentation

Flag: Critical study for SIDS endpoint

23-JAN-2004 (50)

Type: flow through
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes

LC50: ca. 173

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, reagent grade

Remark: 10% Mortality in control in the test with the smaller fishes (mean animal weight 1.8 g, mean total length 5.9 cm).
Result: The tests showed no effect of different ammonium salts on mortality (ammonium chloride, ammonium bicarbonate, amonium hydrogen phosphate, or ammonium sulfate). For ammonium sulfate, the LC50 mean range at pH 7.89-7.94 was determined as 0.764-0.921 mg un-ionized NH3/L (=36.7-39.2 mg total ammonia-N/L), for young fish of mean weights of 1.8 and 2.1 g weight. Overall, susceptibility to ammonia decreased as the fish developed from sac fry to juveniles, and increased hereafter.
Test condition: Two 96-hour tests were run with ammonium sulfate within a series of 86 flow-through tests, ranging in duration from 96 hours to 35 days with the aim to investigate ammonia toxicity and to compare effects of different ammonium salts. Five test tanks and a control were used for each test. Fish were acclimated to the tanks for at least 2 days, except for 5 tests with 1-day acclimation periods.
The two tests with ammonium sulfate were performed with each 10 fish/tank, mean animal weight 1.8 and 2.1 g, mean total length 5.9 and 6.2 cm at pH 7.89 and 7.94, respectively. Mean temperatures for the experiment reported were 12.4 and 12.5 deg C.
Tanks had a water flow rate of 500 mL every 2-3 minutes; replacement time was about 5 hours, and full concentration was reached within 18 hours.
Reliability: (2) valid with restrictions
Limited documentation.
Flag: Critical study for SIDS endpoint
22-JUL-2004 (51)

Type: static
Species: other: Heteropneustes fossilis (water catfish)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**

Result: The result obtained with the logistic calculation method was a 96-hour LC50 of 3760.8 mg/L (95% confidence limits: 3270.9, 4324.2).
The calculation using the Spearman-Karber estimation resulted in a 96-hour LC50 value of 3531.7 mg/L (confidence limits: 3052.9, 4085.7).
Reliability: (2) valid with restrictions
limited documentation
23-AUG-2005 (52)

Type: semistatic
Species: Brachydanio rerio (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50: = 250

Method: other: see Test Condition
GLP: no
Test substance: other TS: ammonium sulfate, analytical grade

Result: LC50 values reported for 24, 48, 72 hours were 430, 340, and 290 mg/L, respectively.
Test condition: The chemical was tested at different concentrations (60, 80, 100, 120, 150, 170, 190, 240, 300 ppm), selected from the results of a preliminary test. In each concentration, 10 fishes were kept and were observed at short intervals during the first day. Dead specimens were removed as soon as possible.
Every 24 hours, all remaining fishes in an aquarium were carefully transferred to a freshly prepared solution.
Temperature and pH were regularly measured and samples from the test aquaria were taken at intervals for chemical analysis.
Reliability: (2) valid with restrictions
limited documentation
Flag: Critical study for SIDS endpoint
26-JAN-2004 (53) (54)

Type: semistatic
Species: Lebistes reticulatus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: = 592
Limit Test: no

Method: other: see Test Condition
Year: 1987
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Remark: 1 ppm was converted to 1 mg/l as no other information was presented.
Result: No mortality occurred at 300 ppm even after 7 days. Complete mortality occurred at 900 ppm. Toxicity symptoms included a high metabolic rate, reddish gills due to hemorrhage, and sluggish movement. The toxicit of ammonium sulfate depends on the concentration of free un-ionized ammonia, which tends to increase in alkaline medium.
No change towards increased toxicity was observed with prolonged exposure times.
Test condition: Fish (mature and gravid females) were acclimated for 10 days in 10-L glass aquaria at pH 8.1-8.4 and 18.5-23.5 °C. Fish were exposed at 20 °C to
ascending concentrations of ammonium sulfate (300 to 900 ppm) in batches of 10. Solutions were renewed on alternate days.
Mortality, defined as median tolerance limits (TLm), were determined at 24, 48, 72 and 96 hours.
Reliability: (2) valid with restrictions
limited documentation
Flag: Critical study for SIDS endpoint
26-JAN-2004 (55)

Type: semistatic
Species: other: Heteropneustes fossilis

Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50: = 2000

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, purity 99%

Result: The LC50 was determined as 2000 mg/.
Test condition: The 96 hour median LC50 was estimated following trimmed Spearman Karber method with 5% trimming (Hamilton et al., 1977) and 24 hour renewal bioassay system prior to commencement of the experiments to the experiments investigating the changes in the respiratory epithelium. No further experimental details are reported on the LC50 experiments.
For the investigations on the respiratory epithelium, 5 groups of ten fish each were exposed to 50 L of 200 mg/L of ammonium sulfate prepared in tap water with 6 mg/L dissolved oxygen at pH 7.5 and a temperature of 26 +/- 2 °C. Parallel controls under similar conditions but without the addition of ammonim sulfate were also maintained. Media were renewed after every 24 hours. Feeding was allowed for a period of 2 hours on every alternate day before the renewal of the media.
Five experimental and five control fish were sacrificed after 5, 10, 20, 30 and 45 days of exposure and 1 cm long fragments of the accessory repiratory organ were fixed and 6 micron thick paraffin sections were stained with Ehrlich´s hematoxylin/eosin, and with various histochemical methods for different carbohydrate moieties.
Reliability: (2) valid with restrictions
limited documentation of results
23-AUG-2005 (56)

Type: static
Species: Alburnus alburnus (Fish, estuary)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: = 310
Limit Test: no

Method: other: see Test Condition
Year: 1979
GLP: no
Test substance: other TS: ammonium sulfate, purity 99.5%

Result: Result: 96-hour LC50 = 310 mg/L (95% confidence limits: 274, 347 mg/L).
Test condition: Fish (about 8 cm from Baltic Sea) were not fed during the test period. The tests were carried out in at least six concentrations and one control, with 10 fish at each concentration. Fish were acclimated for at least two weeks prior to the experiments and fed once a day until 48 hours prior to the tests.
Water (salinity 7 o/oo) for the fishwas maintained at pH 7.8 (not observed during the test) and 10 °C. The fish were tested in 60-liter of brackish water in 70-liter aquaria.
Reliability: (2) valid with restrictions

limited documentation
Flag: Critical study for SIDS endpoint
26-JAN-2004 (57) (58)

Type: flow through
Species: other: Sciaenops ocellatus (Red Drum, a warmwater marine fish), early life stage test
Exposure period: 10 day(s)
Unit: **Analytical monitoring:** yes
LC50: = 27
Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, no further details

Remark: 1. Nominal concentrations of ammonium sulfate were used as the source of ammonium and ammonia in the experiments. The results reported as ammonia concentrations have been calculated from the total ammoniacal N using the formulas of Whitfield as modified by Bower and Bidwell (1979). (See: C.E. Bower and J.P. Bidwell (1979). Ionization of ammonia in seawater: effects of temperature, pH, and salinity. Journal of the Fisheries Research Board of Canada 35, 1012-1016.) The LC50 value reported here has been back-converted, using linearly extrapolated conversion ratios derived from the nearest available tabulated entries for total ammoniacal nitrogen and ammonia, and not from a back conversion using the equations of Bower and Bidwell. The error caused by this procedure is less than 4%.
2. Once lethal concentrations were established for eggs and first-feeding larvae, three week old postlarvae were evaluated for 1 week for changes in ammonia toxicity with age and development. Post larvae were less sensitive than newly hatched larvae. Whereas a concentration of 7.7 mg N L-1 (calculated 0.55 mg/L ammonia) killed all newly hatched larvae within 1 week, a slightly higher exposure was tolerated by 3 week old fish.
Result: The 96 hour LC50 (95% confidence limits) was 5.6 (4.2 - 7.4) mg/L total N (approximately converted from 0.39 (0.29 - 0.53) mg ammonia per litre, as calculated by the method of Bower and Bidwell.)
Test condition: TEST ORGANISMS: Eggs were obtained from laboratory spawnings induced by manipulations of photoperiod and temperature cycles. Fertilised red drum eggs were exposed to controlled concentrations of ammonium sulfate in static tests that were maintained for up to 14 days. Culture methods are described in Holt et al (1981). (Holt, J., Godbout, R., and C. R. Arnold (1991) Effects of temperature and salinity on egg hatching and larval survival of red drum Sciaenops ocellatus. United States National Marine Fisheries Service Fishery Bulletin 79, 569-573.)
TEST VESSEL: The test was a static test.PHYSICAL/CHEMICAL PARAMETERS: The pH of the seawater used varied from 8.0 to 8.2. Salinity varied from 2.8 to 3.2%. Background concentrations of un-ionized ammonia were 0.001-0.018 mg/L. deg C temperature was maintained at either 25 or 26 deg C +/- 0.5 0C. Dissolved oxygen varied from 5.4 to 6.4 mg/L.
TEST CONDITION: Three replicates of each concentration and

of the control contained an average of 65 eggs each. Eggs hatched within 24 hours, and larvae began to feed on the third day post hatch. Mortality was assessed daily. Experiments with older fish included 3 to 4 replicates with 6 to 10 fish each. Concentrations were 0.2, 1.5, 3.6, 4.5, 7.7, 20.0, 100.0, and 500.0 total nitrogen, measured with a Gilford spectrometer. Concentrations were adjusted throughout the exposure to keep them within 10% of the test doses.The 96 hour median lethal concentrations were calculated by the method of Litchfield and Wilcoxon. Treatment means were compared with the controls by Dunnetts test with a 5% significance level.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
23-AUG-2005 (27)

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: > 100

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, reagent-grade, no further details

Remark: In this study, 27 commercial inorganic and organic chemicals were tested simultaneously in seven aquatic species.
Result: A LC50 value could not be determined because the proportion killed at the highest dose level (100 mg/L) was less than 50%. No further details reported.
Test condition: TEST ORGANISMS: Fathead minnow (pimephales promelas), 0.2 - 0.5 g. The test organisms were acclimated to the control diluent water in the breeding/rearing tanks (time period not reported). Food was withheld for the 24 hours preceding start of the test. Juveniles as uniform in size as possible were collected from the colonies. Ten juvenile organisms were exposed to the test chemical in each treatment. Biological loading was kept below 0.5 gram wet weight per liter of test solution. The average wet weight of a randomly chosen set of minnows was determined at the start of the test.

TEST VESSEL: The assay was performed in seamless glass, 30.5-cm cuboidal, Pyrex chromatographic jars to which 20 liters of test solution was added.

TEST SOLUTION: The test chemical was added directly to the diluent water to give a nominal concentration of 100 mg/L. The test chemical concentrations were not analyzed. Once the test solutions were prepared, the starting temperature, dissolved oxygen and pH values were determined for each exposure concentration. When the starting pH of the test soluton fell outside the extremes of 6.5 to 8.5, the pH was adjusted to 7.0 by the addition of 10% (v/v) NaOH or 10% (v/v) H2SO4.

DILUTION WATER: Water quality was routinely monitored to characterize the diluent water and ensure its suitability according to the standard method for the examination of

water and wastewater, 16th ed., American Public Health Association, Washington DC pp. 689-832. The chemical characteristics are reported in the publication and are in accordance with standards required by current testing guidelines. Activated carbon-filtered, dechlorinated and tempered industrial service water from Lake Ontario was used in all tests.

PHYSICAL/CHEMICAL PARAMETERS:
Determination of the temperature, dissolved oxygen and pH of each test solution were made in conjunction with the daily biological observation. The test temperature target was 20 +/- 1 °C. If the dissolved oxygen concentration in a test chamber fell below 40% of the starting level in a test, the test was repeated with 0.05 L/min glass-sparger aeration All tests were conducted within the extremes of 6.5 to 8.5 pH units. The photoperiod duration was 16 h of light. The air-water interface of each tank received approximately 50 ft-c of cool-white fluorescent light.

BIOLOGICAL PARAMETERS: biological observations were made daily. Survival, condition and behavioural information were recorded. Dead organism were removed when observed. If more than one-half of the population exposed in any treatment was found to be dead, additional aquaria containing lower concentrations of test soution were set up.

EXPOSURE PERIOD: 96 hours.

STATISTICAL METHOD: The 96-h LC50 value was derived using an interpolation method as described by Stephan (Stephan CE: Methods for calculating an LC50: Aquatic toxicology and hazard evaluation. In: FL Mayer and JL Hamelink, eds., STP 634. American Society for Testing and Materials, Philadelphia, PA, pp. 65-84). The linear interpolation uses the logarithm transformation of the concentration and the angle transformation of the percent dead between the two doses that bracket 50% response.

TEST CONCENTRATIONS:
100, 10, 1 and 0.1 mg/l

YEAR OF STUDY: not reported.

Reliability: (2) valid with restrictions
concentration not measured. Limited documentation of results.

Flag: Critical study for SIDS endpoint

26-JAN-2004 (59)

Type: static
Species: other: Agonus cataphractus (armed bullhead, hooknose, pogge), seawater fish
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no

Method: other: see Test Conditions
GLP: no
Test substance: other TS: ammonium sulfate, analytical grade

Result: 96-h LC50 (September 1977): 210 mg/L,
96-h LC50 (December 1977): 200 mg/L
96-h LC50 (June 1978):130 mg/L

Test condition: The fish were caught in the estuary of the River Crouch by the Laboratory`s research vessel using a modified 2 m beam trawl with 10 mm mesh in the cod end. On arrival in the laboratory the animals were tranferred to polyethylene stock tanks at a maximum density of 100 fish per tank.
Water at the test emperature of 15 °C was used to slowly fill the tanks and any dead or injured animals were removed. The animals were maintained in well aerated, gently flowing sea water for 2-4 days before the start of the test. They were not fed during their period in the laboratory, which never exceeded 9 days.
Test tanks were filled with 10 L of sea water and aerated for at least 1 hour. Ten fish of 50-100 mm total length (about 2-8 g) were then randomly added to each tank. Diseased animals were excluded. A further acclimatisation period of 2 hour was allowed before the test solutions were added.
Test solutionswere made up by gently stirring a measured amount of the well mixed test substance into the sea water in the test tanks.
Ammonium sulfate was added to the test tanks as 10% w/v solution in distilled water. Four concentrations were set up initially, with controls of clean sea water. Duplicates were used at each concentration.
The concentrations chosen started at 10,000 uL/L (1%) and decreased at half-logarithmic intervals (ie 3300, 1000 and 330 uL/L).
Test solutions were renewed daily to discard metabolites and counteract losses of the test substance due to absorption by the test organisms, degradation or volatilization.
The dissolved oxygen concentration in each test tank was measured and recorded daily. If the dissolved oxygen concentration in any of the test tanks dropped below 70% air saturation value then the test was repeated with additional aeration.
The pH of the test solution was measured and recorded 1 and 24 hours after the start of the test, but is not reported in the paper. The intended pH range was between pH 6 and pH 8.
Each test continued for 96 hours.
The tanks were inspected at frequent intervals, including 24, 48, 72 and 96 hours after adding the test solution, and dead animals (defined as those not responding to gentle prodding) were recorded and removed. At the time of each observation and for each tank the cumulative percentage mortality was calculated. The time for 50% lethality (LT50) and the LC50s were determined. 95% confidence limits on the LT50 were calculated according to the method of Litchfield (1949).
The three tests with ammonium sulfate were carried out in September and December 1977, June 1978.

Reliability: (2) valid with restrictions
limited documentation, nominal concentrations; pH not given.

Flag: Critical study for SIDS endpoint

26-JAN-2004 (60)

Type: static

Species: other: Oreochomis mossambicus (Peters) (order: Cypriniformes)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no data

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, commercial grade

Result: LC50 values depended on temperature:
20 deg C: 330 mg/l/96 h
25 deg C: 175 mg/l/96 h
30 deg C: 89.3 mg/l/96 h
35 deg C: 75.5 mg/l/96 h.
At 30 and 25 deg C, fish exhibited adverse clinical signs when exposed to sublethal concentrations. After 72 hours of exposure, fish showed erratic swimming movement, rapid opercular movement, heavy mucus secretion over body, rupture of buccal epithelium as evidenced by the presence of blood clots and respiratory distress.
Test condition: Fingerlings of Oreochomis Mossambicus (15 +/- 2.5 g) were collected from local ponds and acclimated to the laboratory condition for 7 days. The 96-hour bioassays were performed in different seasons of the year under four temperautres (20, 25, 30, and 35 deg C), using well water. two to 4 fishes in each jar and 8 to 16 fishes per concentration were used. LC50 values were determined by the method of Litchfield and Wilcoxon. Year of study: not reported.
Reliability: (2) valid with restrictions
limited documentation
23-AUG-2005 (61)

Type: static
Species: other: fishes Labeo rohita, Catla catla, Cirrhinus mrigala, Cyprinus carpio, Tilapia mossambica
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, commercial grade

Remark: The values are reported in ppm units, und were converted to mg/L (1 ppm = 1 mg/L).
Result: In the tests with ammonium sulfate the following results were found for fingerlings:

- L. rohita: LC50 67 mg/L (LC5 20 mg/L, LC95 115 mg/L, n=72).
- C. catla: LC50 48 mg/L (LC5 0.0015 mg/L, LC95 120 mg/L, n=84).
- C. mringala: LC50 62 mg/L (LC5 16 mg/L, LC95 109 mg/L, n=134).
- C. carpio: LC50 141 mg/L (LC5 86 mg/L, LC95 218 mg/L, n=80).
- T. mossambica: LC50 50 mg/L (LC5 0.009 mg/L, LC95 131 mg/L, n=190)

Hatchlings were more susceptible to ammonium sulfate than

fingerlings of the same species.
Results from the tests with hatchlings were as follows:

- C. carpio: LC50 77 mg/L (LC5 13 mg/L, LC95 141 mg/L; n=160).
- L. rohita, C. catla, C. mringala: LC50 49 mg/L (LC5 7 mg/L, LC95 85 mg/L; n=146).

Test condition: Fishes were purchased from local farms and were acclimated to the laboratory conditions for 96 hours. The mean lengths and weights of the fishes used were as follows:
- Labeo rohita 48+/- 3.2 mm, 1.634 +/- 0.183 g
- Catla catla 49 +/- 1.5 mm, 3.495 +/- 0.311 g
- Cirrhinus mrigala 24 +/- 1.1 mm, 0.46 +/- 0.046 g
- Cyprinus carpio 41 +/- 1.5 mm, 1.374 +/- 0.076 g
- Tilapia mossambica 42 +/- 2.5 mm, 1.719 +/- 0.197 g.

Hatchlings of major carps and Cyprinus carpio were acclimated for 2 hours to the laboratory conditions.
The experiments were performed in the laboratory at 30.8 +/- 1.2 °C, using unchlorinated borehole water (DO 9.9, total alkalinity 290 ppm as CaCO3, pH 7.5).
Tests were run for 96 hours in 15 L glass aquaria for fingerlings and in 500 mL glass beakers for hatchlings according to the APHA methods (1975). Two to four fishes in each aquarium and 8-16 fishes per concentration, and 25 hatchlings in each beaker were tested.
All tests were repeated five to eight times acompanied with controls.
The following chemicals were tested: urea, ammonium sulfate, superphosphate, muriate of potash, and lime.

Reliability: (2) valid with restrictions
limited documentation

23-AUG-2005 (62)

Type: semistatic
Species: other: Heteropneustes fossilis
Exposure period: 10 day(s)
Unit: mg/l **Analytical monitoring:** no data

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Result: The goblet cells show cyclic increased (due to hyperplasia and hyperactivity) followed by decrease (due to exhaustion and degenration) mucogenic activity. Cyclic hemorrhage takes place due to rupture of the tips of the secondary lamellae, which also regenerate several times. This causes hyperplasia of the haphazardly arranged epithelial cells, leading to decreased secondary lamellar density. Uncomtrolled hyperplasia also causes increased distance of respiratory blood-air barrier, which along with decreased lamellar density results in impaired aerial respiration, leading to asphyxiation and ultimate death of the fish.

Test condition: H. fossilis (length 16 +/- 2 cm) collected locally were acclimated in the laboratory for 20 days in tap water in plastic aquaria. They were regularly fed with minced goat liver. Feeding was discontinued one day before the start of the experiment. Ten groups of 10 fish each, irrespective of their sex, were exposed to 50 L of 2000 ppm of ammonium

sulfate solution prepared in tap water (dissolved oxygen 6 mg/L, water temperature 22 +/- 2 °C, pH 7.8, hardness 23.2 mg/L). In controls, no ammonium sulfate was added. After every 24 hours the media were renewed. Dead fish, if any, were immediately removed. Decrease in concentration of ammonium sulfate between two renewals was not measured. Fragments of the air sac from the anterior end of 2 experimental and 2 control fish wer fixed in 10% neutral formalin and Bouin´s fluid from each of the 6 h, 12 h, 1 d, 2 d, 3 d, 4 d, 6 d, 8 d, and 10 d treated specimens. Six micron thick paraffin sections as well as whole mounts were stained with Ehrlich´s hematoxylin/eosin, and with various histochemical methods for different carbohydrate moieties.

Reliability: (2) valid with restrictions
limited documentation

23-JAN-2004 (63)

Type: semistatic
Species: other: Heteropneustes fossilis
Exposure period: 45 day(s)
Unit: mg/l **Analytical monitoring:** no data
LOEC : = 200

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Result: Density and dimension of the goblet mucous cells of the outer opercular epidermis increased markedly in the initial stages of exposure. Perinuclear vacuoles appeared in the necrotic epithelial cells which also bear pyknotic nuclei before their shedding at several stages of treatment. The club cells also exhibited great vacuolization. The damage became more extensive in later stages of exposure when severe wear and tear of the epidermis took place. The inner opercular lining however did not show such massive necrotic changes. Hyperplasia of the epithelial cells and great vacuolization at various stages of exposure were the main histopathological alterations.

Test condition: Histopathological analysis of the sublethal toxicity induced by 200 mg/L (10% of the 96-hour LC50 value) to the outer and inner opercular epidermis was performed.
TEST ORGANISMS: Healthy individuals of H. fossilis (length 16-18 cm, body weight 35-40 g) collected from a single population at Varanasi were acclimated in large plastic aquaria for 3 weeks. Fish were fed with minced goat liver on every alternate day. Water was renewed after every 24 hours, leaving no fecal matter and unconsumed food. For histopathological analysis, five groups of ten fish each were exposed separately to 50 L of 200 mg/L [10% of the 96-hour LC50 value determined by trimmed Spearman-Karber (with 5% trimming) method and 24 hours renewal bioassay sytems] ammonium sulfate solution prepared in tap water having pH 7.5, dissolved oxygen 6 mg/L, water hardness 23.2 mg/L and water temperature 22 +/- 22 °C. In the appropriate control groups, no ammonium sulfate was added. Experimental and control media were renewed after every 24 hours. Feeding was allowed for control and experimental groups for 3 hours before the renewal of the media. Five experimental and five

control fish each were sacrificed after 5, 10, 20, 30, and 45 days of exposure. Opercula were fixed in 10% neutral formalin, Bouin`s fluid and Helly`s fluid. Six µm paraffin sections were stained with Ehrlich`s hematoxylin/eosin for routine histopathological analysis, periodic acid Schiff (PAS) for neutral glycoproteins, alcian blue pH 2.0 for sulfated mucopolysachharides, alcian blue pH 2.5 for acidic glycoproteins, alcian blue 2.5/PAS dual staining for neutral and acidic glycoproteins and bismarck brown for water-resistant mucoproteins. Morphometric measurements were taken using an oculometer and stage micrometer. Standard statistical procedures based on random sampling from 10 different sections from all the five fish of each stage of all the experimental and control groups were performed. One way analysis of variance (ANOVA) followed by Duncan`s multiple range test were done for multiple comparison. Since the differences between the measurements taken from various control groups at different time intervals of the exposure were not significant, the average of all the control groups was taken into consideration.

Reliability: (2) valid with restrictions
limited documentation

02-FEB-2004 (64)

Type: static
Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, commercial grade

Result: 96-hour LC50 values for various life stages of C. carpio at different temperatures:

20 °C: egg 70 mg/L, spawn 101 mg/L, fry 120 mg/L.
24 °C: egg 67 mg/L, spawn 124 mg/L, fry 140 mg/L.
28 °C: egg 60 mg/L, spawn 78 mg/L, fry 93 mg/L.
32 °C: egg 18 mg/L, spawn 52 mg/L, fry 121 mg/L.
36 °C: egg 23 mg/L, spawn 48 mg/L, fry 45 mg/L.
After six days following eyposure, all embryos died within the sac. No abnormal development exhibited to other temperatures. Organ formation was not observed.
The results indicated that eggs were more sensitive to ammonium sulfate than spawn and fry at all temperatures. The authors surmised that the inhibitory effects of ammonium sulfate on the development of eggs at high temperatures may be due to the interaction of several metabolites like ammonia nitrate and nitrite with various enzymes in the embryo.

Test condition: Spawn and fry (0.8 +/- 0.02 g) of C. carpio were acclimated to laboratory conditions for 7 days. Eggs were collected from an unfertilized pond. Five temperatures within the natural temperature range of streams and ponds in India were used in the study.
The experiments were performed for 96 hours in 500 mL glass beakers according to the APHA (1975) methods, using unchlorinated tubewell water (DO 7.5 ppm, total alkalinity 200 ppm as CaCO3, pH 7.5).

Five eggs in each container and 40 spawn per concentration were used. Same methods were employed for spawn and fry of fish. Tests were repeated six times accompanied with ocntrols. No death of eggs, spwan and fry were observed in controls.

Reliability: (2) valid with restrictions
limited documentation, concentration range not reported
02-FEB-2004 (65)

Type: static
Species: Ictalurus punctatus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l **Analytical monitoring:** yes
Limit Test: no

Method: other: similar to those suggested by the USEPA (1975)
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Result: When compared on the basis of un-ionized ammonia nitrogen (NH3-N), ammonium chloride solutions were more toxic than ammonium sulfate solutions. Median lethal concentrations (24h- LC50s) at pH 8.8, 8.0, 7.2 and 6.0 were, respecitvely, 1.91, 1.45, 1.04, and 0.74 mg NH3-N/L for ammonium chloride, and 2.24, 1.75, 1.16, and 0.81 mg NH3-N/L for ammonium sulfate. Ionized ammonia (NH4) was not lethal at concentrations up to 1787 mg NH4-N/L.
The author conclude that based on acute toxicity tests that account for osmotic effects, NH4+ must be considered an essentially nontoxic substance. The apparent increase in toxicity of NH3 to channel catfish at lower pH is due to osmotic effects of toxicant formulations, rather than specifically to NH4.
Test condition: 8 static 24-h median lethal tests were conducted with 16-g channel catfish. Fish were acclimated for at least 2 weeks prior to testing. Feeding was stopped one week before use in tests (minimize ammonia excretion by the fishes).
The 110-Liter aquaria held 80 liters of 21 °C water at various pHs (6.0, 7.2, 8.0, 8.8). Each exposure group consisted of 10 fish. Buffers were added and fish acclimated for 24 hours before ammonia (as ammonium sulfate or ammonium chloride) was added.
Reliability: (2) valid with restrictions
limited documentation; 24-hour LC50 tests used.
23-JAN-2004 (66)

Type: static
Species: Oncorhynchus gorbuscha (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50: ca. 770

GLP: no data

Result: Late alevins (completion of yolk absorption) were the most sensitive and had the lowest 96-hour LC50 values. Concentrations as low as 1.2 ppb reduced fry weight in the 61 day exposures. Only levels > 10 ppb stimulated early

emergence of immature fry.

Test condition: Three types of tests were conducted:
(1) Eyed eggs, alevins, and fry were exposed in separate tests to short term, high concentrations of ammonia (> 50 ppb) in static systems to determine the sensitivity of each early life stage.
(2) Alevins were exposed at different developmental stages to low concentrations of ammonia (< 3 ppb) in flow-through systems for up to 61 days to determine the effect of long-term exposures on size of emerging fry.
(3) Alevins were exposed to high concentrations of ammonia (30-150 ppb) in flow-through systems for 24 hours to determine whether ammonia would cause emergence of immature fry.
Eggs, alevins, and fry were also exposed to static solutions of ammonium sulfate for 96 hours in freshwater at pH of 6.3-6.5 and 3.7-4.8 °C. Each test group consisted of 25 individuals.

Reliability: (2) valid with restrictions
limited documentation

02-FEB-2004 (28)

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 10 day(s)
Unit: mg/l **Analytical monitoring:**

Method: other
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Result: The NOEC for the juvenile fish, which were 6 weeks old at the start of the 10 day test, was 66.6 mg/L ammonium nitrogen. The corresponding LOEC was 118.0 mg/L ammonium nitrogen.
The NOEC for the juvenile fish, which were 9 weeks old at the start of the 10 day test, was 134.0 mg/L ammonium nitrogen. The corresponding LOEC was 227.0 mg/L ammonium nitrogen.

Test condition: TEST ORGANISMS: Pimephales promelas were hatched and reared in a laboratory colony in the EPA laboratory facilities at Corvallis, Ore. They were reared in aquaria with a continuous flow of temperature controlled fresh water whose temperature was gradually increased from the collection temperature of ca. 10 0C to the test temperature of 20 +/- 1 deg C. Temperature was continuously recorded. They were held at a photoperiod of 14:10 light:dark. The fish were fed newly hatched brine shrimp and then frozen fish food.
TEST VESSEL: The test species were exposed to ammonium sulfate in 18 10-L aquaria with a continuously-flowing water diluter system that automatically delivered six concentrations of chemical and controls to three aquaria per concentration.
PHYSICAL/CHEMICAL PARAMETERS: Rearing and test water was obtained from wells near the Willamette River at Corvallis, Oregon. Dissolved oxygen, measured by electrode, was maintained near saturation by the flowing water. pH (median value 7.3) was measured by electrode. Total hardness (72 + 4.2 mg/L), alkalinity (63 + 2.8 mg/L), and conductivity

(188.8 + 7.3 µS/cm) were measured by EPA methods 130.2, 310.1, and 120.1, respectively. Background ammonium-N ranged from 0.005 - 0.010 mg/L in well water, and from 0.00 to 0.03 mg/L in control tanks. Water samples for measurement of ammonium sulfate were taken from each concentration (each aquarium in the diluter) on four days, and analysed with a Hach DR/700 digital photometer. Quality assurance techniques were used in the analysis.

TEST CONDITION: Five 6-week old juveniles were used in each of three replicate aquaria, and the test was run for 10 days. Length and mean wet weight were measured at the end of the test. The means and standard deviations of the ammonium-nitrogen concentrations measured at the 5 experimental concentrations and in the control were 211.2 +/- 17.5, 118.0 +/- 9.8, 66.6 +/- 7.7, 37.0 +/- 4.6, 17.4 +/- 3.8 and 0 mg/L ammonium nitrogen, respectively. Ten day LOEC values and NOEC values were determined with the Dunnetts multiple comparison procedure.

Test substance: ammonium sulfate, 99.2% pure (Reagent Grade, Mallinckrodt Baker Inc, Phillipsburg, NJ)

Reliability: (1) valid without restriction

22-JUL-2004 (67)

Type: static
Species: Poecilia reticulata (Fish, fresh water)
Exposure period: 120 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC0: = 200
LC50: = 395
LC100: = 600
LC5 : = 218

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, analytical grade

Remark: Original reference in foreign language
Test condition: Acute toxicity tests were performed with various materials used as fertilizer in Czechoslovakia.
The tests were performed at pH 7.5 to 7.8 at 19-22 degree C with Poecilia reticulata Peters. No detail is provided as to the experimental procedure (no. of test animals, exposure etc.).

Reliability: (4) not assignable
Abstract, original reference in foreign language

22-JUL-2004 (68)

Type: static
Species: Salmo salar (Fish, fresh water, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** yes

Method: other: see Test Condition
GLP: no
Test substance: other TS: commercial sample

Result: 96-hour LC50 (mean pH 6.05 and mean temperature 12.5-17.1 °C) = 0.091 - 0.111 mg un-ionized NH3-N/L.

LC50 values increased from 0.031 to 0.111 mg un-ionized NH3-N/L at mean pH 6.0 as temperature increased from 2.1 to 17.1 °C.
Similarly, at mean pH 6.45, mean LC50 increaed from 0.030 to 0.146 mg NH3-N/L as temperature increased from 1.8 to 12.5°C.
The behaviour of intoxicated fish consisted of coughing, hyperventilation followed by sporadic ventilation, twisting, loss of equilibrium and spiral swimming, convulsions and death following a coma-like state.

Test condition: The fish were acclimated for at least 2 weeks to the control water quality and stocking conditions before being use in experiments. Each aquarium had 10 fish. The tests were conducted in twelve 40-L glass aquaria filled with 30-L of test solution. The fish were not fed during temperature acclimation (24 hours) and the 96-hours test period. The pH was adjusted using NaOH or H2SO4.
The 96-hour LC50 values were determined for various pH (6.00 - 6.45) and temperatures (1.8 - 17.8 °C). The resulting LC50 values (603-2758 mg/L) expressed in ammonium sulfate concentrations, have been converted from 128 - 585 mg/L TAN given in the report.

Reliability: (2) valid with restrictions
limited documentation

23-JAN-2004 (69)

Type: static
Species: other: Barbus ambassis (freshwater Barb)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50: = 545
Limit Test: no

Method: other: see Test Condition
GLP: no
Test substance: other TS: ammonium sulfate, not further specified

Remark: Cited as ppm (1 ppm was converted to 1 mg/L)

Result: No mortality occurred in controls. dissolved oxygen in all the tanks was above 5 mg/L and the pH varied from 7.5 to 7.6.
No mortality occurred within 96 hours test period at 400 ppm ammonium sulfate. However, concentrations higher than these were found to be toxic.

Test condition: Fishes were kept in the laboratory at room temperature for a fortnight. Eight to ten fishes measuring about 4.5 cm in length were used in each container. Replicate 96-hour static bioassays were conducted in fresh water using five different concentrations and two controls were run simultaneously. Observations were made at 24, 48, 72, and 96 hours after the beginning of the test. Experiments were carried out at a controlled temperature of 28 deg C +/- 1 deg C. During the experiments, dissolved oxygen, pH, nitrite and nitrate were determined using standard procedures. LC50 values were estimated by plotting the percentage mortality at 24- and 48-hours for each concentration and reading the estimated concentraiton lethal to 50% of the fish from the graph.
Year of study: not reported.

Reliability: (2) valid with restrictions
limited documentation

23-JAN-2004 (70)

Type: static
Species: other: Labeo umbratus (fam. Cyprinidae)

Result: Effects on hematological parameters were reported when adult fish were treated with sublethal concentrations (no value reported) of ammonium sulfate for 72 hours. The lethal dose was quoted to be 420 mg/L, a concentration at which mortality ocurred within 4 hours. Sublethal doses caused statistical significant changes such as an increase in the number of white blood cells dimension, and a decrease

in MCV, and average corpuscular hemoglobin (ACH) when compared to controls. Other parameters such as hematocrit, number of red blood cells, hemoglobin and total plasma proteins were decreased and MCHC increased, however without statistical significance.
Reliability: (2) valid with restrictions
26-JAN-2004 (71)

Species: other: Tilapia mossambica
Exposure period: 192 hour(s)

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, commercial grade

Result: At 26 and 30°C a significant decrease in feeding rate was observed at concentrations >100 ppm. No effect on feeding rate was noted at a temperature of 20°C.
Test condition: Fish from local hatcheries. Tests were run at 3 different temperatures: 20, 26, and 30°C in November/December, February/March and June/July, respectively. Two fish per aquarium and eight fish per concentration were exposed to three temperature conditions separately. Live earthworm were cut into pieces and placed into each aquarium every 24 hours and any uneaten food was removed at the same time. The number of pieces consumed by fish were observed and recorded. Tests were run for 192 hours. Nine concentrations of ammonium sulfate were used (10, 20, 40, 60, 80, 100, 120, 140, 160 ppm).
Reliability: (2) valid with restrictions
limited documentation
26-JAN-2004 (72)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: flow through
Species: Daphnia magna (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50 : > 100

Method: other: see Test Conditions
GLP: no data
Test substance: other TS: ammonium sulfate, reagent-grade, no further details

Remark: In this study, 27 commercial inorganic and organic chemicals were tested simultaneously in seven aquatic species.

Result: A LC50 value could not be determined because the proportion killed at the highest dose level (100 mg/L) was less than 50%. No further details reported.

Test condition: TEST ORGANISMS: Water flea (Daphnia magna) of first and second larval instar. The test organisms were acclimated to the control diluent water in the breeding/rearing tanks (time period not reported). Food was withheld for the 24 hours preceding start of the test. Juveniles as uniform in size as possible were collected from the colonies. Ten juvenile organisms were exposed to the test chemical in each treatment. Biological loading was kept below 0.5 gram wet weight per liter of test solution.

TEST VESSEL: The assay was performed in seamless glass, 30.5-cm cuboidal, Pyrex chromatographic jars to which 20 liters of test solution was added. The test organisms were segregated in welded stainless steel, 55-mesh wirecloth baskets (5.5 cm in diameter x 7.5 cm in depth). Each basket was suspended from a 1-rpm motor-driven mechanism that raised and lowered the baskets in the water column. A stainless steel band, slotted every 0.5 cm, facilitated the positioning of the baskets so that the submerged volumes changed from one-third to wo-thirds during each cycle. One-half of the volume of the sumerged basket was exchanged with the main tank volume every minute.

TEST SOLUTION: The test chemical was added directly to the diluent water to give a nominal concentration of 100 mg/L. The test chemical concentrations were not analyzed. Once the test solutions were prepared, the starting temperature, dissolved oxygen and pH values were determined for each exposure concentration. When the starting pH of the test soluton fell outside the extremes of 6.5 to 8.5, the pH was adjusted to 7.0 by the addition of 10% (v/v) NaOH or 10% (v/v) H2SO4.

DILUTION WATER: Water quality was routinely monitored to characterize the diluent water and ensure its suitability according to the standard method for the examination of water and wastewater, 16th ed., American Public Health Association, Washington DC pp. 689-832. The chemical characteristics are reported in the publication and are in accordance with standards required by current testing guidelines. Activated carbon-filtered, dechlorinated and tempered industrial service water from Lake Ontario was used in all tests.

PHYSICAL/CHEMICAL PARAMETERS:
Determination of the temperature, dissolved oxygen and pH of each test solution were made in conjunction with the daily biological observation. The test temperature target was 20 +/- 1 °C. If the dissolved oxygen concentration in a test chamber fell below 40% of the starting level in a test, the test was repeated with 0.05 L/min glass-sparger aeration All tests were conducted within the extremes of 6.5 to 8.5 pH units. The photoperiod duration was 16 h of light. The air-water

interface of each tank received approximately 50 ft-c of cool-white fluorescent light.

BIOLOGICAL PARAMETERS: biological observations were made daily. Survival, condition and behavioural information were recorded. Dead organism were removed when observed. If more than one-half of the population exposed in any treatment was found to be dead, additional aquaria containing lower concentrations of test soution were set up.

EXPOSURE PERIOD: 96 hours.
TEST CONCENTRATION: 100, 10, 1 and 0.1 mg/l

STATISTICAL METHOD: The 96-h LC50 value was derived using an interpolation method as described by Stephan (Stephan CE: Methods for calculating an LC50: Aquatic toxicology and hazard evaluation. In: FL Mayer and JL Hamelink, eds., STP 634. American Society for Testing and Materials, Philadelphia, PA, pp. 65-84). The linear interpolation uses the logarithm transformation of the concentration and the angle transformation of the percent dead between the two doses that bracket 50% response.

YEAR OF STUDY: not reported.

Reliability: (2) valid with restrictions
concentration not measured. Limited documentation of results.

Flag: Critical study for SIDS endpoint

26-JAN-2004 (59)

Type: flow through
Species: Asellus intermedius (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50 : > 100

Method: other: see Test Conditions
GLP: no
Test substance: other TS: ammonium sulphate, reagent-grade, no further details

Remark: In this study, 27 commercial inorganic and organic chemicalswere tested simultaneously in seven aquatic species.
Result: A LC50 value could not be determined because the proportion killed at the highest dose level (100 mg/L) was less than 50%. No further details reported.
Test condition: TEST ORGANISMS: Pillbug (Asellus intermedius), 0.012 g. The test organisms were acclimated to the control diluent water in the breeding/rearing tanks (time period not reported). Food was withheld for the 24 hours preceding start of the test. Juveniles as uniform in size as possible were collected from the colonies. Ten juvenile organisms were exposed to the test chemical in each treatment. Biological loading was kept below 0.5 gram wet weight per liter of testsolution.

TEST VESSEL: The assay was performed in seamless glass, 30.5-cm cuboidal, Pyrex chromatographic jars to which 20 liters of test solution was added. The test organisms were

segregated in welded stainless steel, 55-mesh wirecloth baskets (5.5 cm in diameter x 7.5 cm in depth). Each basket was suspended from a 1-rpm motor-driven mechanism that raised and lowered the baskets in the water column. A stainless steel band, slotted every 0.5 cm, facilitated the positioning of the baskets so that the submerged volumes changed from one-third to wo-thirds during each cycle. One-half of the volume of the sumerged basket was exchanged with the main tank volume every minute.

TEST SOLUTION: The test chemical was added directly to the diluent water to give a nominal concentration of 100 mg/L. The test chemical concentrations were not analyzed. Once thetest solutions were prepared, the starting temperature, dissolved oxygen and pH values were determined for each exposure concentration. When the starting pH of the test soluton fell outside the extremes of 6.5 to 8.5, the pH was adjusted to 7.0 by the addition of 10% (v/v) NaOH or 10% (v/v) H2SO4.

DILUTION WATER: Water quality was routinely monitored to characterize the diluent water and ensure its suitability according to the standard method for the examination of water and wastewater, 16th ed., American Public Health Association, Washington DC pp. 689-832. The chemical characteristics are reported in the publication and are in accordance with standards required by current testing guidelines. Activated carbon-filtered, dechlorinated and tempered industrial service water from Lake Ontario was used in all tests.

PHYSICAL/CHEMICAL PARAMETERS:
Determination of the temperature, dissolved oxygen and pH of each test solution were made in conjunction with the daily biological observation. The test temperature target was 20 +/- 1 °C. If the dissolved oxygen concentration in a test chamber fell below 40% of the starting level in a test, the test was repeated with 0.05 L/min glass-sparger aeration All tests were conducted within the extremes of 6.5 to 8.5 pH units. The photoperiod duration was 16 h of light. The air-water interface of each tank received approximately 50 ft-c of cool-white fluorescent light.

BIOLOGICAL PARAMETERS: biological observations were made daily. Survival, condition and behavioural information were recorded. Dead organism were removed when observed. If more than one-half of the population exposed in any treatment was found to be dead, additional aquaria containing lower concentrations of test soution were set up.

EXPOSURE PERIOD: 96 hours.
TEST CONCENTRATION: 100, 10, 1 and 0.1 mg/l

STATISTICAL METHOD: The 96-h LC50 value was derived using an interpolation method as described by Stephan (Stephan CE: Methods for calculating an LC50: Aquatic toxicology and hazard evaluation. In: FL Mayer and JL Hamelink, eds., STP 634. American Society for Testing and Materials, Philadelphia, PA, pp. 65-84). The linear interpolation uses

the logarithm transformation of the concentration and the angle transformation of the percent dead between the two doses that bracket 50% response.

YEAR OF STUDY: not reported.

Reliability: (2) valid with restrictions
concentration not measured. Limited documentation of results.

Flag: Critical study for SIDS endpoint

26-JAN-2004 (59)

Type: flow through
Species: Gammarus fasciatus (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50 : > 100

Method: other: see Test Conditions
GLP: no
Test substance: other TS: ammonium sulphate, reagent-grade, no further details

Remark: In this study, 27 commercial inorganic and organic chemicals were tested simultaneously in seven aquatic species.

Result: A LC50 value could not be determined because the proportion killed at the highest dose level (100 mg/L) was less than 50%. No further details reported.

Test condition: TEST ORGANISMS: Sideswimmer (Gammarus fasciatus), 0.007 g. The test organisms were acclimated to the control diluent water in the breeding/rearing tanks (time period not reported). Food was withheld for the 24 hours preceding start of the test. Juveniles as uniform in size as possible were collected from the colonies. Ten juvenile organisms were exposed to the test chemical in each treatment. Biological loading was kept below 0.5 gram wet weight per liter of test solution.

TEST VESSEL: The assay was performed in seamless glass, 30.5-cm cuboidal, Pyrex chromatographic jars to which 20 liters of test solution was added. The test organisms were segregated in welded stainless steel, 55-mesh wirecloth baskets (5.5 cm in diameter x 7.5 cm in depth). Each basket was suspended from a 1-rpm motor-driven mechanism that raised and lowered the baskets in the water column. A stainless steel band, slotted every 0.5 cm, facilitated the positioning of the baskets so that the submerged volumes changed from one-third to wo-thirds during each cycle. One-half of the volume of the sumerged basket was exchanged with the main tank volume every minute.

TEST SOLUTION: The test chemical was added directly to the diluent water to give a nominal concentration of 100 mg/L. The test chemical concentrations were not analyzed. Once the test solutions were prepared, the starting temperature, dissolved oxygen and pH values were determined for each exposure concentration. When the starting pH of the test soluton fell outside the extremes of 6.5 to 8.5, the pH was adjusted to 7.0 by the addition of 10% (v/v) NaOH or 10% (v/v) H2SO4.

DILUTION WATER: Water quality was routinely monitored to characterize the diluent water and ensure its suitability according to the standard method for the examination of water and wastewater, 16th ed., American Public Health Association, Washington DC pp. 689-832. The chemical characteristics are reported in the publication and are in accordance with standards required by current testing guidelines. Activated carbon-filtered, dechlorinated and tempered industrial service water from Lake Ontario was used in all tests.

PHYSICAL/CHEMICAL PARAMETERS:
Determination of the temperature, dissolved oxygen and pH of each test solution were made in conjunction with the daily biological observation. The test temperature target was 20 +/- 1 °C. If the dissolved oxygen concentration in a test chamber fell below 40% of the starting level in a test, the test was repeated with 0.05 L/min glass-sparger aeration All tests were conducted within the extremes of 6.5 to 8.5 pH units. The photoperiod duration was 16 h of light. The air-water interface of each tank received approximately 50 ft-c of cool-white fluorescent light.

BIOLOGICAL PARAMETERS: biological observations were made daily. Survival, condition and behavioural information were recorded. Dead organism were removed when observed. If more than one-half of the population exposed in any treatment was found to be dead, additional aquaria containing lower concentrations of test soution were set up.

EXPOSURE PERIOD: 96 hours.
TEST CONCENTRATION: 100, 10, 1 and 0.1 mg/l.

STATISTICAL METHOD: The 96-h LC50 value was derived using an interpolation method as described by Stephan (Stephan CE: Methods for calculating an LC50: Aquatic toxicology and hazard evaluation. In: FL Mayer and JL Hamelink, eds., STP 634. American Society for Testing and Materials, Philadelphia, PA, pp. 65-84). The linear interpolation uses the logarithm transformation of the concentration and the angle transformation of the percent dead between the two doses that bracket 50% response.

YEAR OF STUDY: not reported.

Reliability: (2) valid with restrictions
concentration not measured. Limited documentation of results.

Flag: Critical study for SIDS endpoint

26-JAN-2004 (59)

Species: Crangon crangon (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: ca. 380 - 600
Limit Test: no

Method: other: see Test Conditions
GLP: no
Test substance: other TS: ammonium sulfate, analytical grade

Remark: EC50(96h) = 380 mg/L (September 1977), 600 mg/L (December 1977), 480 mg/L (June 1978), 400 mg/L (September 1978), 540 mg/L (March 1979)

Test condition: The animals were caught in the estuary of the River Crouch by the Laboratory`s research vessel using a modified 2 m beam trawl with 10 mm mesh in the cod end. On arrival in the laboratory the animals were tranferred to polyethylene stock tanks at a maximum density of 200 shrimps per tank.
Water at the test emperature of 15 °C was used to slowly fill the tanks and any dead or injured animals were removed.
The animals were maintained in well aerated, gently flowing sea water for 2-4 days before the start of the test. They were not fed during their period in the laboratory, which never exceeded 9 days.
Test tanks were filled with 10 L of sea water and aerated for at least 1 hour. 20 shrimps of 40-70 mm (about 1-3 g) were then randomly added to each tank. Diseased animals were excluded. A further acclimatisation period of 2 hours was allowed before the test solutions were added.
Test solutionswere made up by gently stirring a measured amount of the well mixed test substance into the sea water in the test tanks.
Ammonium sulfate was added to the test tanks as 10% w/v solution in distilled water. Four concentrations were set up initially, with controls of clean sea water. Duplicates were used at each concentration.
The concentrations chosen started at 10,000 uL/L (1%) and decreased at half-logarithmic intervals (ie 3300, 1000 and 330 uL/L).
Test solutions were renewed daily to discard metabolites and counteract losses of the test substance due to absorption by the test organisms, degradation or volatilization.
The dissolved oxygen concentration in each test tank was measured and recorded daily. If the dissolved oxygen concentration in any of the test tanks dropped below 70% air saturation value then the test was repeated with additional aeration.
the pH of the test solution was measured and recorded 1 and 24 hours after the start of the test, but is not given in the paper. The intended pH range was between 6 and 8.
Each test continued for 96 hours.
The tanks were inspected at frequent intervals, including 24, 48, 72 and 96 hours after adding the test solution, and dead animals (defined as those not responding to gentle prodding) were recorded and removed. At the time of each observation and for each tank the cumulative percentage mortality was calculated. The time for 50% lethality (LT50) and the LC50s were determined. 95% confidence limits on the LT50 were calculated according to the method of Litchfield (1949).
The three tests with ammonium sulfate were carried out in September and December 1977, June and September 1978, and March 1979.

Reliability: (2) valid with restrictions
Limited documentation, no analytical monitoring; pH not reported.

Flag: Critical study for SIDS endpoint

02-FEB-2004 (60)

Type: flow through
Species: other aquatic worm: Dugesia tigrina
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50 : > 100

Method: other: see Test Conditions
GLP: no data
Test substance: other TS: ammonium sulphate, reagent-grade, no further details

Remark: In this study, 27 commercial inorganic and organic chemicals were tested simultaneously in seven aquatic species.
Result: A LC50 value could not be determined because the proportion killed at the highest dose level (100 mg/L) was less than 50%. No further details reported.
Test condition: TEST ORGANISMS: Flatworm (Dugesia tigrina, Platyhelminthes), 0.006 g. The test organisms were acclimated to the control diluent water in the breeding/rearing tanks (time period not reported). Food was withheld for the 24 hours preceding start of the test. Juveniles as uniform in size as possible were collected from the colonies. Ten juvenile organisms were exposed to the test chemical in each treatment. Biological loading was kept below 0.5 gram wet weight per liter of test solution.

TEST VESSEL: The assay was performed in seamless glass, 30.5-cm cuboidal, Pyrex chromatographic jars to which 20 liters of test solution was added. The test organisms were segregated in welded stainless steel, 55-mesh wirecloth baskets (5.5 cm in diameter x 7.5 cm in depth). Each basket was suspended from a 1-rpm motor-driven mechanism that raised and lowered the baskets in the water column. A stainless steel band, slotted every 0.5 cm, facilitated the positioning of the baskets so that the submerged volumes changed from one-third to wo-thirds during each cycle. One-half of the volume of the sumerged basket was exchanged with the main tank volume every minute.

TEST SOLUTION: The test chemical was added directly to the diluent water to give a nominal concentration of 100 mg/L. The test chemical concentrations were not analyzed. Once the test solutions were prepared, the starting temperature, dissolved oxygen and pH values were determined for each exposure concentration. When the starting pH of the test soluton fell outside the extremes of 6.5 to 8.5, the pH was adjusted to 7.0 by the addition of 10% (v/v) NaOH or 10% (v/v) H2SO4.

DILUTION WATER: Water quality was routinely monitored to characterize the diluent water and ensure its suitability according to the standard method for the examination of water and wastewater, 16th ed., American Public Health Association, Washington DC pp. 689-832. The chemical characteristics are reported in the publication and are in accordance with standards required by current testing guidelines. Activated carbon-filtered, dechlorinated and tempered industrial service water from Lake Ontario was used in all tests.

PHYSICAL/CHEMICAL PARAMETERS:
Determination of the temperature, dissolved oxygen and pH of each test solution were made in conjunction with the daily biological observation. The test temperature target was 20 +/- 1 °C. If the dissolved oxygen concentration in a test chamber fell below 40% of the starting level in a test, the test was repeated with 0.05 L/min glass-sparger aeration All tests were conducted within the extremes of 6.5 to 8.5 pH units. The photoperiod duration was 16 h of light. The air-water interface of each tank received approximately 50 ft-c of cool-white fluorescent light.

BIOLOGICAL PARAMETERS: biological observations were made daily. Survival, condition and behavioural information were recorded. Dead organism were removed when observed. If more than one-half of the population exposed in any treatment was found to be dead, additional aquaria containing lower concentrations of test soution were set up.

EXPOSURE PERIOD: 96 hours.
TEST CONCENTRATION: 100, 10, 1 and 0.1 mg/l.

STATISTICAL METHOD: The 96-h LC50 value was derived using an interpolation method as described by Stephan (Stephan CE: Methods for calculating an LC50: Aquatic toxicology and hazard evaluation. In: FL Mayer and JL Hamelink, eds., STP 634. American Society for Testing and Materials, Philadelphia, PA, pp. 65-84). The linear interpolation uses the logarithm transformation of the concentration and the angle transformation of the percent dead between the two doses that bracket 50% response.

YEAR OF STUDY: not reported.

Reliability: (2) valid with restrictions
concentration not measured. Limited documentation of results.

Flag: Critical study for SIDS endpoint

26-JAN-2004 (59)

Type: static
Species: other: Perna Viridis (green mussel, seawater)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50 : = 94.9

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, reagent-grade, no further details

Remark: The pH variation over the experiment, from pH 8.125 at 5 mg/L NH4+ to pH 7.85 at 75 mg/L NH4+ is small, and a slightly smaller percentage of unionised ammonia will be present at the higher concentration level. Thus the experiment will correctly reflect the toxicity of the ionised ammonium salt.

Result: The 96 hour LC50 was 94.9 mg ammonium sulfate N/l (converted from 13.0 mg NH4+ L-1); 5% confidence limit 12.5 - 14.0 mg/NH4/l.

The 48 hour LC50 was 113,15 mg ammonium sulfate N L-1 (converted from 15.5 mg NH4+/l); 5% confidence limit 13.75 - 16.0 mg NH4+/l. The LC50 values, computed from the mortality rates, show that 50% of the organisms died within 49.8 hours at 15.0 mg NH4+/l, and that 50% died after 30 h at 75 mg NH4+/l.

Test condition: TEST ORGANISMS: The green mussel Perna viridis was collectedfrom an unpolluted region at Someswara rocky shore (12047'N;74051'E). All individuals were obtained during low tide from the sub-tidal belt. They were transported to the laboratory and kept unfed in large polythene trays, in aerated sea water for 24 hours before the beginning of the experiments. Young mussels in the size range 20 to 24 mm were used, and only members of the same population were examined in a single set of experiments.
TEST VESSELS: Cylindrical glass troughs of 5 L capacity containing 4 l of sea water were used.
TEST METHODOLOGY: Ten individuals were exposed to each concentration of NH4+, at 0, 5, 10, 15, 20, 50, and 75 mg /L. Mortality tests were conducted over 96 hours. Death was defined as the inability to close the valves upon mechanical stimulus, and as valve gaping of 5mm. Dead individuals were removed from the media at 12 hour intervals.
PHYSICAL/CHEMICAL PARAMETERS: The pH of the sea water varied between 8.0 and 8.3, and the temperature of the sea water varied between 28°C and 30°C during the experiments. In the presence of ammonium, the pH varied from pH 8.125 at 5 mg/L NH4+ to pH 7.85 at 75 mg/L NH4+.

Reliability: (2) valid with restrictions
Concentrations not measured. Limited documentation of results, and of the ammonium sulfate used in the experiment.

Flag: Critical study for SIDS endpoint

23-AUG-2005 (73)

Type: flow through
Species: Daphnia magna (Crustacea)
Exposure period: 120 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 272
EC100: = 500
EC5 : = 110

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, analytical grade

Test condition: Acute toxicity tests were performed with various materials used as fertilizer in Czechoslovakia.
The tests were performed at pH 7.5 to 7.8 at 19-22 degree C with Daphnia magna Strauss. No detail is provided as to the experimental procedure (no. of test organisms, exposure etc.).
Test concentration: 100, 10, 1 and 0.1 mg/l

Reliability: (4) not assignable
Abstract, original reference in foreign language

26-JAN-2004 (68)

Type: static
Species: Daphnia pulicaria (Crustacea)

Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: = 39.4

Method: other: see Test Condition
GLP: no
Test substance: other TS: Fire-Trol 100 (ammonium sulfate with n attapulgite clay thickener, no further details)

Result: for formulation
EC50 = 62 mg/l
Test condition: Static tests wer conducted in reconstituted water with a pH of 7.4 (pH of Fire-Trol-solution: 6.9 to 7.1).
TEST ORGANISM: gammarus pseudolimnaeus (scud).
Test substance: Fire-Trol (= 63.5 % (NH4)2SO4
Reliability: (2) valid with restrictions
basic data are given
26-JAN-2004 (74)

Type: static
Species: other aquatic mollusc
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data

Method: other: see Test Conditon
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Remark: Following 48 hour exposure, the concentrations of ammonium sulfate killing 100% of freshwater snails were 1250 mg/L and 1000 mg/L for the adults of Helisoma trivolvis and of Biomphalaria havanensis, respectively. 48-hour LC50 values were 700 and 491 mg/L for Heliosoma trivovis and Biomphalaria havanensis, respectively.
Results indicate 24 hour LC50 values of 558 mg/L and 669 mg/L for eggs, 393 mg/L and 526 mg/L for juveniles, and 701 mg/L and 657 mg/L for adults of Helisoma trivolvis and Biomphalariahavanensis, respectively.
Test condition: Eggs, juveniles or adults of the two species were exposed for 24 or 48 hours. Details of the method were referred to a companion paper.
Reliability: (2) valid with restrictions
limited documentation
22-JUL-2004 (75)

Type: flow through
Species: other aquatic mollusc: Helisoma trivolvis
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
LC50 : > 100

Method: other: see Test Conditions
GLP: no data
Test substance: other TS: ammonium sulphate, reagent-grade, no further details

Remark: In this study, 27 commercial inorganic and organic chemicals were tested simultaneously in seven aquatic species.
Result: A LC50 value could not be determined because the proportion

killed at the highest dose level (100 mg/L) was less than 50%. No further details reported.

Test condition: TEST ORGANISMS: Snail (Helisoma trivolvis), 0.180 g. The test organisms were acclimated to the control diluent water in the breeding/rearing tanks (time period not reported). Food was withheld for the 24 hours preceding start of the test. Juveniles as uniform in size as possible were collected from the colonies. Ten juvenile organisms were exposed to the test chemical in each treatment. Biological loading was kept below 0.5 gram wet weight per liter of test solution.

TEST VESSEL: The assay was performed in seamless glass, 30.5-cm cuboidal, Pyrex chromatographic jars to which 20 liters of test solution was added. The test organisms were placed directly in the aquaria.

TEST SOLUTION: The test chemical was added directly to the diluent water to give a nominal concentration of 100 mg/L. The test chemical concentrations were not analyzed. Once the test solutions were prepared, the starting temperature, dissolved oxygen and pH values were determined for each exposure concentration. When the starting pH of the test soluton fell outside the extremes of 6.5 to 8.5, the pH was adjusted to 7.0 by the addition of 10% (v/v) NaOH or 10% (v/v) H2SO4.

DILUTION WATER: Water quality was routinely monitored to characterize the diluent water and ensure its suitability according to the standard method for the examination of water and wastewater, 16th ed., American Public Health Association, Washington DC pp. 689-832. The chemical characteristics are reported in the publication and are in accordance with standards required by current testing guidelines. Activated carbon-filtered, dechlorinated and tempered industrial service water from Lake Ontario was used in all tests.

PHYSICAL/CHEMICAL PARAMETERS:
Determination of the temperature, dissolved oxygen and pH of each test solution were made in conjunction with the daily biological observation. The test temperature target was 20 +/- 1 °C. If the dissolved oxygen concentration in a test chamber fell below 40% of the starting level in a test, the test was repeated with 0.05 L/min glass-sparger aeration All tests were conducted within the extremes of 6.5 to 8.5 pH units. The photoperiod duration was 16 h of light. The air-water interface of each tank received approximately 50 ft-c of cool-white fluorescent light.

BIOLOGICAL PARAMETERS: biological observations were made daily. Survival, condition and behavioural information were recorded. Dead organism were removed when observed. If more than one-half of the population exposed in any treatment was found to be dead, additional aquaria containing lower concentrations of test soution were set up.

EXPOSURE PERIOD: 96 hours.
TEST CONCENTRATION: 100, 10, 1 and 0.1 mg/l

STATISTICAL METHOD: The 96-h LC50 value was derived using aninterpolation method as described by Stephan (Stephan CE: Methods for calculating an LC50: Aquatic toxicology and hazard evaluation. In: FL Mayer and JL Hamelink, eds., STP 634. American Society for Testing and Materials, Philadelphia, PA, pp. 65-84). The linear interpolation uses the logarithm transformation of the concentration and the angle transformation of the percent dead between the two doses that bracket 50% response.

YEAR OF STUDY: not reported.

Reliability: (2) valid with restrictions
concentration not measured. Limited documentation of results.

26-JAN-2004 (59)

Type: static
Species: other aquatic mollusc: Lamellidens marginalis
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** no data
LC50 : = 3528

Method: other: static test as described in Doudoroff (1951), Sewage Wastes 23, 130
Year: 1995
Test substance: other TS: ammonium sulfate, purity 99%

Test condition: TEST ORGANISMS: Freshwater mussels, belonging to the species Lamellidens marginalis weighing 30 +/- 2 g, collected from the ponds of an Indian village. The animals were fed ad libitum on freshwater plankton and acclimated to laboratory conditions for a period of 15 days.
Concentration (sublethal): 10 and 176.4 mg/l about 7 days

EXPOSURE TO TEST SUBSTANCE: Batches of 10 animals were exposed to different concentrations of ammonium sulfate ranging from 1,000 to 5,000 mg/L with an interval of 100 mg/L. Each experiment in the selected concentrations of ammonium sulfate was repeated six times.
OBSERVATIONS: After 48 hours the number of animals died at each concentration was recorded. the animal was considered dead when the mantle edge was no longer responsive to touch and failure to close the shell valves.
DETERMINATION OF THE LC50: The mortality in each concentration was taken to determine the LC50 by graphical plots of per cent mortality, probit mortality against log concentration of ammonium sulfate according to the method of Finney, 1964.

Reliability: (2) valid with restrictions
limited documentation

26-JAN-2004 (76)

Type: static
Species: other aquatic worm: Schistosoma mansoni
Exposure period: 4 hour(s)
Unit: **Analytical monitoring:** no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, from Sigma Chemical company

Remark: 1) The ammonium sulfate concentrations studied here are much higher than those which would be applied in the field. Thus ammonium sulfate addition is not a practical way to control S. Mansoni infestation.2) The LC5, LC50, and LC95 values given below are as reported. However, the 6 hour values are effected by mortality in the controls, due to the aging population, and several of the other values given are outside the range of the test concentrations used (0, 0.1, 0.2, 0.3, 0.4, and 0.5% ammonium sulfate.) Thus only the 4 hour LC50 value of 0.16% is recommended.

Result: A 1% solution of ammonium sulfate was sufficient to completely inhibit hatching of S. Mansoni eggs. The LC5, LC50, and LC95 values for miracidial survival were 0.07%, 0.80%, and 10.61% after 2 hours of exposure; 0.03%, 0.16%, and 0.90% after 4 hours of exposure, and 0.30, 0.20, and 0.40% after 6 hours of exposure.
The 4-hr LC50 was determined as 0.16% ammonium sulfate

Test condition: TEST ORGANISMS: The life cycle of a Puerto-Rican strain of Schistosoma mansoni has been maintained in the Schistosomiasis Labotatory of the Tulane University School of Public Health and Tropical Medicine, using Biomphalaria glabrata, NIH albino strain, as intermediate hosts and laboratory mice as definitive hosts. Schistosome eggs and miracidia were obtained from the livers of infected mice, which were removed by disection and homogenised at a low speed for 20-30 seconds using a Waring blender. The homogenate was poured into a flask for 20-30 minutes settlement, after which the supernatant was carefully poured off. The egg-containing sediment was used in the hatchability and survival studies.
HATCHABILITY STUDIES: After resuspending and thoroughly mixing the egg-containing sediment in 8-10 ml of 0.85% NaCl, 1.0 ml aliquots were transferred to each of several 100 ml volumetric flasks which were then filled with the specified dilutions of ammonium sulfate. Three replicates were made for each ammonium sulfate concentration. The flasks, except for the necks, were wrapped with aluminium foil and were then exposed to light. The negatively geotactic and the positively photoactic behaviour of the miracidia caused them to aggregate in the necks. Thirty minutes later, the upper 5 ml were removed from the neck of each flask, and all the miracidia present were killed with iodine and counted. The percentages hatching were obtained by comparing the number of miracidia obtained in each test concentration to that obtained in the control with no added ammonium sulfate.
SURVIVAL STUDIES: The egg-containing sediment obtained from the homogenate was rinsed into a 1 l sidearm flask, previously covered, except for the sidearm, with black cloth. The flask was then filled with dechlorinated water. A bright light was nest directed toward the uncovered portion of the flask. The negatively geotactic and the positively photoactic behaviour of the miracidia caused them to aggregate in the sidearm, after about 15 minutes. They were then removed from the sidearm with a pasteur pipette and transferred to wells of the test chamber, a laboratory

tissue culture plate which was used with a disecting microscope. A stock solution of ammonium sulfate was freshly prepared, and used for further dilutions. 4 to 6 miracidia were picked up in 1 ml dechlorinated water, and placed in each well of the test chamber. 1 ml of double strength ammonium sulfate solution was then added,according to the test schedule, giving a total of 2 ml in each experimental well. 6 Replicate concentrations were employed. The test concentrations were 0 (control), 0.1, 0.2, 0.3, 0.4, and 0.5% ammonium sulfate. Careful hourly observations were made under the disecting microscope. Motionless miracidia (round shaped in the control group and elongated in the experimental group) were recognised as dead.
STATISTICAL ANALYSIS: The EPA Probit statistical package (EPA Probit Analysis Program Version 1.3) as used to calculate LC5, LC50, and LC95 values after 2, 4, and 6 hours of exposure.

Reliability: (2) valid with restrictions
Only the LC50 result at 4 hours is statistically robust. Concentrations not measured. Limited documentation of results.

22-MAY-2003 (77)

Species: other aquatic worm: planaria tigrina
Exposure period: 120 hour(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 97
EC100: = 150
EC5 : = 41

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, analytical grade

Test condition: Acute toxicity tests were performed with various materials used as fertilizer in Czechoslovakia.
The tests were performed at pH 7.5 to 7.8 at 19-22 degree C with Planaria tigrina Girard. No detail is provided as to the experimental procedure (no. of test organisms, exposure etc.).

Reliability: (4) not assignable
Abstract, original reference in foreign language

26-JAN-2004 (68)

Type: static
Species: other: Belostoma sp. (aquatic insect)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: commercial grade ammonium sulphate containing 20.6% nitrogen.

Remark: Inconsistent information in test methodology described - between the number of animals per vessel and the total number of animals used in the test - for a valid testing

regime.
Result: An LC50 value of 181 ppm was observed at 22.0 °C.
An LC50 value of 63 ppm was observed at 32.5 °C.
Test condition: TEST ORGANISMS: Test organisms were collected from local ponds and acclimated to laboratory conditions for 10 to 96 hours. TEST VESSEL: 500 ml glass beakers containing unchlorinated borehole water (pH 7.5; DO 7.6 ppm; total alkalinity 190 ppm as CaCO3; ammonia nitrogen 0.05ppm) TEST METHODOLOGY: Twenty animals were kept per vessel, and four replicates of each test were conducted, along with controls. Bioassays were conducted after the method of APHA, AWWA, and WPCF (1971): Standard method for the examination of water and wastewater, 13th Ed. APHA Inc, New York, 874. 96 hour tests were carried out for insects and molluscs. Test temperatures were 22.0 + 1.1 0C and 32.5 + 1.3 0C. 500 organisms were used in this test, a similar test using another substance, and the controls. The LC50 and LC 95 values were determined by probit analysis.

Reliability: (3) invalid
26-JAN-2004 (78)

Type: static
Species: other: Biomphalaria alexandria (freshwater snails)
Exposure period: 168 hour(s)
Unit: mg/l **Analytical monitoring:** no
EC50: < 1000
Limit Test: no

Method: other: see Test Condition
Test substance: other TS: ammonium sulfate, no further details

Remark: From observation of figure 1 in the paper, the LC 50 value will lie between 400 and 600 ppm ammonium sulfate.
Result: Quoting the paper "1000 ppm of ammonium sulfate caused 100% mortality." "Results in this study give some support to the suggestion of Tchounwou (referred to as "Tchounwou, B.P., Englande, J. R., and Malek, E.A.: Arch. Environ. Contam. Toxicol. 21, 359(1991)") who reported that in very dilute solution, the toxicity of certain fertilizers may be attributed to the formation of unionized ammonia, while in high concentrations of the compounds rapid mortality may be due to the activity of the sol
Test condition: TEST ORGANISMS: Biomphalaria alexandria snails were obtained from Abu-Rawash, Giza Governate, Egypt, and were maintained in the laboratory under suitable conditions of aeration, temperature (250C), and feeding. Other test conditions as given in reference M. I. Nassar and A. Z. Abdel-hamid (1993). Phytochemical and Molluscidial screening of some local plants. Bull. NRC, Egypt, Vol 18 (4), pp 335-341.) EXPOSURE PERIOD: 7 days.
Reliability: (3) invalid
Time of the LC50 test not explicitely stated in this paper. pH not measured. Ionic strength not measured. Concentrations not measured. Very limited documentation of results. Secondary quotation.
26-JAN-2004 (79)

Type: static
Species: other: Biomphalaria alexandria (freshwater snails)
Exposure period: 168 hour(s)
Unit: mg/l **Analytical monitoring:** no
Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, no further details

Result: 100 ppm ammonium sulfate reduced the mean number of eggs per snail by 59.8% from the number observed in the control group. Observation of figures 2 and 3 in the paper shows that ammonium sulfate had some effect on both hatchability (more than 50% reduction after 10 days) and growth (aproximately 33% reduction after 14 days).
Test condition: TEST ORGANISMS: Biomphalaria alexandria snails were obtained from Abu-Rawash, Giza Governate, Egypt, and were maintained in the laboratory under suitable conditions of aeration, temperature (250C), and feeding. Other test conditions as given in reference M. I. Nassar and A. Z. Abdel-hamid (1993). Phytochemical and Molluscidial screening of some local plants. Bull. NRC, Egypt, Vol 18 (4), pp335-341.).
TEST METHODOLOGY:50 adult snails were selected for similarity of size and diameter were treated with 100 ppm ammonium sulfate or left untreated (control group). Snails were left to deposit their eggs over a period of 2 weeks. Egg masses were collected, counted, and kept under suitable conditions for hatching.
HATCHABILITY: Newly-hatched snails were counted daily until they reached 14 days of age.GROWTH RATE: The shell diameters of newly hatched snails was measured daily.
Reliability: (3) invalid
Time of the initial exposure not explicitely stated in this paper. pH not measured. Ionic strength not measured. Concentrations not measured.
26-JAN-2004 (79)

Type: static
Species: other: Cyclops viridis (Zooplankton)
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: commercial grade ammonium sulphate containing 20.6% nitrogen

Result: An LC50 value of 130 ppm was observed at 22.0 °C.
An LC50 value of 65 ppm was observed at 32.5 °C.
Test condition: TEST ORGANISMS: Test organisms were collected from local ponds and acclimated to laboratory conditions for 10 to 96 hours.
TEST VESSEL: 500 ml glass beakers containing unchlorinated borehole water (pH 7.5; DO 7.6 ppm; total alkalinity 190 ppm as CaCO3; ammonia nitrogen 0.05ppm) TEST METHODOLOGY: Twenty

animals were kept per vessel, and four replicates of each test were conducted, along with controls. Bioassays were conducted after the method of APHA, AWWA, and WPCF (1971): Standard method for the examination of water and wastewater, 13th Ed. APHA Inc, New York, 874. 72 hour tests were carried out for zooplankton. Test temperatures were 22.0 + 1.1 °C and 32.5 + 1.3 °C. 1600 organisms were used in this test, a similar test using another substance, and the controls. The LC50 and LC 95 values were determined by probit analysis.

Reliability: (4) not assignable
Documentation insufficient for assessment. pH not measured. Ammonium sulfate fertiliser characterisation is very limited. Concentrations not measured. Number of concentrations used and nominal concentration values not stated. Limited documentation of results.

22-MAY-2003 (78)

Type: static
Species: other: Diaptomus sp. (Zooplankton)
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: commercial grade ammonium sulphate containing 20.6% nitrogen

Result: An LC50 value of 151 ppm was observed at 22.0 °C.
An LC50 value of 45 ppm was observed at 32.5 °C.

Test condition: TEST ORGANISMS: Test organisms were collected from local ponds and acclimated to laboratory conditions for 10 to 96 hours.
TEST VESSEL: 500 ml glass beakers containing unchlorinated borehole water (pH 7.5; DO 7.6 ppm; total alkalinity 190 ppm as CaCO3; ammonia nitrogen 0.05ppm)
TEST METHODOLOGY: Twenty animals were kept per vessel, and four replicates of each test were conducted, along with controls. Bioassays were conducted after the method of APHA, AWWA, and WPCF (1971): Standard method for the examination of water and wastewater, 13th Ed. APHA Inc, New York, 874. 72 hour tests were carried out for zooplankton. Test temperatures were 22.0 + 1.1 °C and 32.5 + 1.3 °C. 1800 organisms were used in this test, a similar test using another substance, and the controls. The LC50 and LC 95 values were determined by probit analysis.

Reliability: (4) not assignable
Documentation insufficient for assessment. pH not measured. Ammonium sulfate fertiliser characterisation is very limited. Concentrations not measured. Number of concentrations used and nominal concentration values not stated.Limited documentation of results.

18-MAY-2003 (78)

Type: static
Species: other: Dragon fly nymph (aquatic insect)
Exposure period: 96 hour(s)

Unit: mg/l **Analytical monitoring:** no
Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: commercial grade ammonium sulphate containing 20.6% nitrogen.

Remark: Inconsistent information in test methodology described - between the number of animals per vessel and the total number of animals used in the test - for a valid testing regime.
Result: An LC50 value of 180 ppm was observed at 22.0 °C.
An LC50 value of 60 ppm was observed at 32.5 °C.
Test condition: TEST ORGANISMS: Test organisms were collected from local ponds and acclimated to laboratory conditions for 10 to 96 hours.
TEST VESSEL: 500 ml glass beakers containing unchlorinated borehole water (pH 7.5; DO 7.6 ppm; total alkalinity 190 ppm as CaCO3; ammonia nitrogen 0.05ppm).
TEST METHODOLOGY: Twenty animals were kept per vessel, and four replicates of each test were conducted, along with controls. Bioassays were conducted after the method of APHA, AWWA, and WPCF (1971): Standard method for the examination of water and wastewater, 13th Ed. APHA Inc, New York, 874.
96 hour tests were carried out for insects and molluscs.
Test temperatures were 22.0 + 1.1 °C and 32.5 + 1.3 °C. 1150 organisms were used in this test, a similar test using another substance, and the controls. The LC50 and LC 95 values were determined by probit analysis.
Reliability: (4) not assignable
Documentation insufficient for assessment. pH not measured. Ammonium sulfate fertiliser characterisation is very limited. Concentrations not measured. Number of concentrations used and nominal concentration values not stated.Limited documentation of results.
18-MAY-2003 (78)

Type: static
Species: other: Dytiscus sp. (aquatic insect)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: commercial grade ammonium sulphate containing 20.6% nitrogen.

Remark: Inconsistent information in test methodology described - between the number of animals per vessel and the total number of animals used in the test - for a valid testing regime.
Result: An LC50 value of 188 ppm was observed at 22.0 °C.
An LC50 value of 46 ppm was observed at 32.5 °C.
Test condition: TEST ORGANISMS: Test organisms were collected from local ponds and acclimated to laboratory conditions for 10 to 96 hours. TEST VESSEL: 500 ml glass beakers containing

unchlorinated borehole water (pH 7.5; DO 7.6 ppm; total alkalinity 190 ppm as CaCO3; ammonia nitrogen 0.05ppm) TEST METHODOLOGY: Twenty animals were kept per vessel, and four replicates of each test were conducted, along with controls. Bioassays were conducted after the method of APHA, AWWA, and WPCF (1971): Standard method for the examination of water and wastewater, 13th Ed. APHA Inc, New York, 874. 96 hour tests were carried out for insects and molluscs. Test temperatures were 22.0 + 1.1 0C and 32.5 + 1.3 0C. 400 organisms were used in this test, a similar test using another substance, and the controls. The LC50 and LC 95 values were determined by probit analysis.

Reliability: (4) not assignable
Documentation insufficient for assessment. pH not measured. Ammonium sulfate fertiliser characterisation is very limited. Concentrations not measured. Number of concentrations used and nominal concentration values not stated.Limited documentation of results.

18-MAY-2003 (78)

Type: static
Species: other: Indoplanorbus exustus (mussel)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: commercial grade ammonium sulphate containing 20.6% nitrogen

Result: An LC50 value of 248 ppm was observed at 22.0 °C.
An LC50 value of 86 ppm was observed at 32.5 °C.

Test condition: TEST ORGANISMS: Test organisms were collected from local ponds and acclimated to laboratory conditions for 10 to 96 hours.
TEST VESSEL: 500 ml glass beakers containing unchlorinated borehole water (pH 7.5; DO 7.6 ppm; total alkalinity 190 ppm as CaCO3; ammonia nitrogen 0.05ppm).
TEST METHODOLOGY: Twenty animals were kept per vessel, and four replicates of each test were conducted, along with controls. Bioassays were conducted after the method of APHA, AWWA, and WPCF (1971): Standard method for the examination of water and wastewater, 13th Ed. APHA Inc, New York, 874. 96 hour tests were carried out for insects and molluscs. Test temperatures were 22.0 + 1.1 °C and 32.5 + 1.3 °C. 1150 organisms were used in this test, a similar test using another substance, and the controls. The LC50 and LC 95 values were determined by probit analysis.

Reliability: (4) not assignable
Documentation insufficient for assessment. pH not measured. Ammonium sulfate fertiliser characterisation is very limited. Concentrations not measured. Number of concentrations used and nominal concentration values not stated.Limited documentation of results.

18-MAY-2003 (78)

Type: static
Species: other: Indoplanorbus exustus (mussel)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: commercial grade ammonium sulphate containing 20.6% nitrogen.

Result: An LC50 value of 248 ppm was observed at 22.0 °C.
An LC50 value of 86 ppm was observed at 32.5 °C.
Test condition: TEST ORGANISMS: Test organisms were collected from local ponds and acclimated to laboratory conditions for 10 to 96 hours.
TEST VESSEL: 500 ml glass beakers containing unchlorinated borehole water (pH 7.5; DO 7.6 ppm; total alkalinity 190 ppm as CaCO3; ammonia nitrogen 0.05ppm).
TEST METHODOLOGY: Twenty animals were kept per vessel, and four replicates of each test were conducted, along with controls. Bioassays were conducted after the method of APHA, AWWA, and WPCF (1971): Standard method for the examination of water and wastewater, 13th Ed. APHA Inc, New York, 874. 96 hour tests were carried out for insects and molluscs. Test temperatures were 22.0 + 1.1 °C and 32.5 + 1.3 °C. 1200 organisms were used in this test, a similar test using another substance, and the controls. The LC50 and LC 95 values were determined by probit analysis.
Reliability: (4) not assignable
Documentation insufficient for assessment. pH not measured. Ammonium sulfate fertiliser characterisation is very limited. Concentrations not measured. Number of concentrations used and nominal concentration values not stated.Limited documentation of results.
18-MAY-2003 (78)

Type: static
Species: other: Lymnaea leuteola (mussel)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: commercial grade ammonium sulphate containing 20.6% nitrogen.

Result: An LC50 value of 255 ppm was observed at 22.0 °C.
An LC50 value of 76 ppm was observed at 32.5 °C.
Test condition: TEST ORGANISMS: Test organisms were collected from local ponds and acclimated to laboratory conditions for 10 to 96 hours.
TEST VESSEL: 500 ml glass beakers containing unchlorinated borehole water (pH 7.5; DO 7.6 ppm; total alkalinity 190 ppm as CaCO3; ammonia nitrogen 0.05ppm).
TEST METHODOLOGY: Twenty animals were kept per vessel, and four replicates of each test were conducted, along with

controls. Bioassays were conducted after the method of APHA, AWWA, and WPCF (1971): Standard method for the examination of water and wastewater, 13th Ed. APHA Inc, New York, 874. 96 hour tests were carried out for insects and molluscs. Test temperatures were 22.0 + 1.1 °C and 32.5 + 1.3 °C. 1700 organisms were used in this test, a similar test using another substance, and the controls. The LC50 and LC 95 values were determined by probit analysis.

Reliability: (4) not assignable
Documentation insufficient for assessment. pH not measured. Ammonium sulfate fertiliser characterisation is very limited. Concentrations not measured. Number of concentrations used and nominal concentration values not stated. Limited documentation of results.

18-MAY-2003 (78)

Type: static
Species: other: Moina micrura (Zooplankton)
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** no
Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: commercial grade ammonium sulphate containing 20.6% nitrogen.

Result: An LC50 value of 141 ppm was observed at 22.0 °C.
An LC50 value of 32 ppm was observed at 32.5 °C.

Test condition: TEST ORGANISMS: Test organisms were collected from local ponds and acclimated to laboratory conditions for 10 to 96 hours.
TEST VESSEL: 500 ml glass beakers containing unchlorinated borehole water (pH 7.5; DO 7.6 ppm; total alkalinity 190 ppm as CaCO3; ammonia nitrogen 0.05ppm)
TEST METHODOLOGY: Twenty animals were kept per vessel, and four replicates of each test were conducted, along with controls. Bioassays were conducted after the method of APHA, AWWA, and WPCF (1971): Standard method for the examination of water and wastewater, 13th Ed. APHA Inc, New York, 874. 72 hour tests were carried out for zooplankton. Test temperatures were 22.0 + 1.1 °C and 32.5 + 1.3 °C. 1400 organisms were used in this test, a similar test using another substance, and the controls. The LC50 and LC 95 values were determined by probit analysis.

Reliability: (4) not assignable
Documentation insufficient for assessment. pH not measured. Ammonium sulfate fertiliser characterisation is very limited. Concentrations not measured. Number of concentrations used and nominal concentration values not stated.Limited documentation of results.

18-MAY-2003 (78)

Type: static
Species: other: Notonecta sp. (aquatic insect)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no

Limit Test: no

Method: other: see Test Condition
GLP: no data
Test substance: other TS: commercial grade ammonium sulphate containing 20.6% nitrogen.

Remark: Inconsistent information in test methodology described - between the number of animals per vessel and the total number of animals used in the test - for a valid testing regime.
Result: An LC50 value of 154 ppm was observed at 22.0 °C.
An LC50 value of 45 ppm was observed at 32.5 °C.
Test condition: TEST ORGANISMS: Test organisms were collected from local ponds and acclimated to laboratory conditions for 10 to 96 hours.
TEST VESSEL: 500 ml glass beakers containing unchlorinated borehole water (pH 7.5; DO 7.6 ppm; total alkalinity 190 ppm as CaCO3; ammonia nitrogen 0.05ppm) TEST METHODOLOGY: Twenty animals were kept per vessel, and four replicates of each test were conducted, along with controls. Bioassays were conducted after the method of APHA, AWWA, and WPCF (1971): Standard method for the examination of water and wastewater, 13th Ed. APHA Inc, New York, 874. 96 hour tests were carried out for insects and molluscs. Test temperatures were 22.0 + 1.1 °C and 32.5 + 1.3 °C. 800 organisms were used in this test, a similar test using another substance, and the controls. The LC50 and LC 95 values were determined by probit analysis.
Reliability: (4) not assignable
Documentation insufficient for assessment. pH not measured. Ammonium sulfate fertiliser characterisation is very limited. Concentrations not measured. Number of concentrations used and nominal concentration values not stated. Limited documentation of results.
18-MAY-2003 (78)

Type: static
Species: other: Viviparous bengalensis (mussel)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
Limit Test: no

Method: other: Test Conditions
GLP: no data
Test substance: other TS: commercial grade ammonium sulphate containing 20.6% nitrogen.

Result: An LC50 value of 248 mg/L (converted from 248 ppm) was observed at 22.0 °C.
An LC50 value of 77 mg/L (converted from 77 ppm) was observed at 32.5 °C.
Test condition: TEST ORGANISMS: Test organisms were collected from local ponds and acclimated to laboratory conditions for 10 to 96 hours.
TEST VESSEL: 500 ml glass beakers containing unchlorinated borehole water (pH 7.5; DO 7.6 ppm; total alkalinity 190 ppm as CaCO3; ammonia nitrogen 0.05ppm).

TEST METHODOLOGY: Twenty animals were kept per vessel, and four replicates of each test were conducted, along with controls. Bioassays were conducted after the method of APHA, AWWA, and WPCF (1971): Standard method for the examination of water and wastewater, 13th Ed. APHA Inc, New York, 874. 96 hour tests were carried out for insects and molluscs. Test temperatures were 22.0 + 1.1 0C and 32.5 + 1.3 0C. 2000 organisms were used in this test, a similar test using another substance, and the controls. The LC50 and LC 95 values were determined by probit analysis.

Reliability: (4) not assignable
Documentation insufficient for assessment. pH not measured. Ammonium sulfate fertiliser characterisation is very limited. Concentrations not measured. Number of concentrations used and nominal concentration values not stated. Limited documentation of results.

22-MAY-2003 (78)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Chlorella vulgaris (Algae)
Endpoint: other: cell number
Exposure period: 18 day(s)
Unit: mg/l **Analytical monitoring:** no data
EC50: = 2700

Method: other: see Test Condition
GLP: no
Test substance: other TS: ammonium sulfate, not further specified

Remark: In the control a lag-phase could be observed until day 11 (decline of cell number to approx. 10x10e6 cells/mL). Logarithmic growth could be seen from day 11 - 18 (decline of cell number up to appr. 70x10e6 cells/mL). From day 18 - 21 the cell number dropped back to 10x10e6 cells/mL. For this reason the calculation of EC50 is referred to the cell number at day 18.
Growth occurred in all ammonia concentrations (10-1000 mg N/L), although less growth was found in cultures containing either very low (10 mg N/L) or very high (750 and 1000 mg N/L) ammonia concentrations.
Algal growth was accompanied by a decrease in nitrogen content in the medium, indicating that nitrogen removal was due to algal uptake and assimilation. Results demonstrate that C. vulgaris can tolerate high concentrations of ammonia.

Result: In the first about 10 days after start, in all test concentration and in the control a lag phase was observed. Thereafter in the control and some test concentrations an exponential increase in cell number was observed up to day 17 or 18.

Based on data presented in graphics an EC50 (18 d, cell count) of about 2700 mg/l could be calculated with "ToxRat Standard Version 2.09 (Demo)". An EC10 could not be calculated by the statistical program.

There are no data available to calculate the 72-hEC50.

Test condition: STOCK CULTURE: Stock culture of Chlorella vulgaris was kept

aseptically in a commercial Bristol medium (Bold Basal medium,) containing 40 mg N /L as KNO3 and 53 mg P /L as KH2PO4.
TEST MEDIA: The test media were prepared in the same way as the Bristol medium except the nitrate component was replaced by different amounts of ammonium sulfate.

A total of 12 ammonium concentrations were prepared: 0, 10, 20, 40, 50, 60, 80, 125, 250, 500, 750, and 1000 mg N/L. The initial cell density was 1x10e6 cells/mL. The pH values of the culture media were adjusted to 7.0 before algal inoculation. The algae were grown in light-dark cycles of 16-8 hours for 21 days. The algal cell number was determined

at 3 or 4 day intervals.
The pH value was adjusted to 7 +/- 0.2 before algae inoculation and was maintained at neutral pH during the study.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
23-AUG-2005 (80)

Species: other aquatic plant: marine phytoplankton
Endpoint: growth rate
Exposure period: 17 day(s)
Unit: mg/l **Analytical monitoring:**

GLP: no
Test substance: other TS: ammonium sulfate, not further specified

Remark: The results demonstrate that marine phytoplankton is more sensitive to ammonium sulfate than freshwater species, with the marine dinoflagellates Gymnodinium splendens and Gonyaulax polyedra showing growth inhibition in a 17d-test at concentrations of 0.706 mg/L ammonium sulfate (calculated from 150 µg/L NH4-N) in a 17d-test. A reduction in photosyntheseis within 3 hours is also shown for Gymnodinium splendens at 0.471 mg/L ammonium sulfate (calculated from 100 µg /L NH4-N). No significance-levels are reported. A LOEC of 0.706 mg/L and a NOEC of 0.471 mg/L (calculated from 150 and 100 µg/L NH4-N) can be estimated for the endpoint growth reduction for Gymnodinium splendens and Gonyaulax polyedra. Also, a LOEC of 0.471 mg/L and a NOEC of 0.235 mg/L (calculated from 100 and 50 µg/L NH4-N) can be estimated for the endpoint reduction in photosynthesis for Gymnodinium splendens. No information on pH in the test media is given.

Result:

Species	Exposure Time	Effect [mg (NH4)2SO4 / L]
Asterionella japonica	up to 17 days	NOEC > 0.942
Chaetoceros affinis	up to 17 days	NOEC > 0.942
Gymnodinium splendens	up to 17 days	NOEC = 0.471 LOEC = 0.706
Gonyaulax polyedra	up to 17 days	NOEC = 0.471 LOEC = 0.706

Dunaliella sp up to 17 days NOEC > 0.942

Test condition: Ammonium sulfate concentrations consisting of 0, 5, 10, 25, 50, 100, 150, and 200 ug atom NH4-N/L were placed in 1-Liter flasks. A control contained 880 ug atom/L NO3-N. After inoculation, duplicate cultures were incubated in a water bath at 21-22 °C under continuous illumination. Growth was measured at daily or half-daily intervals for up to 17-days. For photosynthesis studies, 90-mL of seawater containing the above concentrations of NH4-N were placed in 125 mL bottles.

Radioactive NaH14CO3 was added and the bottles were incubated for 3 hours.

Reliability: (2) valid with restrictions
Incomplete documentation.

16-JUL-2004 (81)

Species: other aquatic plant: Chondrus crispus Stackhouse (Irish Moss)
Endpoint: other: photosynthesis
Unit: mg/l **Analytical monitoring:**
LOEC: = 46.4

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Remark: Cultivated Chondrus crispus was used in N-NH4 uptake experiments in the laboratory. An elevation of temperature increased the apparent rate of uptake, especially up to 11 deg C. Uptake in the dark was found to be 83% of that in the light. The apparent uptake decreased with increasing internal N pool; rates were 26.5, 22.2 and 20.2 ug N g dry wt-1 min-1 for internal N pools of 2.7, 3.5 and 4.6%, respectively. Apparent uptake increased with the substrate N concentration. The resulting curve has two components: an active uptake and a diffusion component at high (more than 5000 ug N L-1) external N levels. Ks and V max were calculated by deducting the diffusion component from the uptake curve: these were 497 ug N L-1 and 14.4 ug N g dry wt-1 min-1, respectively, and reflect a low substrate affinity.

Result: A concentration of 10 mg N/L (about 46.4 mg (NH4)2SO4 / L) reduced photosynthesis by 12.8% and as much as 30 mg N / L reduced photosynthesis by 34.5%. No other effects were observed.

Test condition: The effect of ammonium sulfate on photosynthesis was tested by following the variations of pH and calculating the corresponding carbon equilibrium as described by Hansson, 1973 (Deep Sea Res. 20, 461-478). N uptake rates were calculated between 5 and 15 minutes into the experiment, normalizing values with the dry weight.
Incubation times varied from 15 minutes (Michaelis-Menten experiments) to 5 hours.

Reliability: (2) valid with restrictions
limited documentation

13-JUL-2004 (82)

Species: other algae:Synechococcus cedrorum

Endpoint: growth rate
Exposure period: 14 day(s)
Unit: mg/l **Analytical monitoring:** no data
LOEC: = 100

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Result: Supplementation of ammonia (from ammonium sulfate) as nitrogen source did not support growth at all. The growth in ammonium sulfate containing medium (50 and 100 mg/L) had a prolonged lag phase, which was followed by a very short exponential and stationary phase, after which the cultures started to turn chlorotic at 14 days of culture.
Test condition: TEST ORGANISMS: unicellular blue-green alga Synechococcus cedrorum IU1191, obtained from the Indiane University Culture collection.
To study the effect of inorganic nitrogen compounds on growth of algae, sodium nitrate, sodium nitrite and ammonium sulfate were used separately in the following concentrations: sodium nitrate: 0.05 g/L and 1.5 g/L (control); sodium nitrite: 0.5 g/L and 0.75 g/L; ammonium sulfate: 0.05 g/L and 0.1 g/L. The cultures were grown under aseptic conditions in Hughe`s medium with sodium nitrite, sodium nitrate or ammonium sulfate as nitrogen source at pH 7.8 and 26 °C with 1400 lux light intensity. The cultures were incubated in side arm flasks (a specially designed conical flask with a side arm or tube), each containing 20 mL of growth medium. Growth was measured by measuring cellular absorbance at 700 nm with a photoelectric colorimeter.
Reliability: (3) invalid
invalid testdesign and insufficient documentation
13-JUL-2004 (83)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: field
Species: other bacteria: nitrogen-fixing cyanobacteria
Unit: **Analytical monitoring:** yes

Method: other: see Test Conditions
GLP: no data
Test substance: other TS: ammonium sulfate as fertilizer, no further details

Remark: The result will be influenced by the pH (4.4) of the soil, which results from ammonium sulfate addition. Increased ammonia volatilisation at acid pH may contribute to the effect.
Result: Applied ammonium sulfate at 17.5, 35, 70, and 140 kg N /ha reduced the numbers of cyanobacteria and the nitrogen fixation observed. However, the ammonium sulfate inputs of up to 70 kg N /ha gave rise to a moderate increase in grain yield, without severely affecting the N2 fixing ability (less than 50% reduction) or cyanobacteria numbers.
Test condition: EXPOSURE PERIOD:
mean of 3 yearly experiments.

SITE DESCRIPTION The rice fields are located in the Eastern Iberian Peninsula, surrounding the coastal lagoon of "La Albufera" near Valencia, Spain. A detailed description of the site and of the rice crop cycle is given in the following references:1) Quesada A, Sánchez Maeso E, and Fernández Valiente E, 1995. New incubation device for in situ measurement of acetylene-reducing activity in ricefields. J. Appl. Phycol. 1, 195-200.2) Quesada A, Sánchez Maeso E, and Fernández Valiente E, 1995. Seasonal variation of chemical properties of rice field soils from Valencia, Spain. Commun. Soil Sci. Plant Anal . 26, 1-19.The text mentions the alkaline condition of soils and sediments.The field trials were conducted in three consecutive crop seasons, using plots of 5m x 20m size, laterally isolated by plastic sheets embedded into the soil at a depth of 10cm. Ammonium sulfate was applied at 0, 17.5, 35, 70, and 140 kg N ha-1. A basal dose of 100 kg/ha P2O5 was supplied, as superphosphate, to all plots. A randomised complete block design with four replicates was employed. Basal P and N doses were applied as a single broadcast application and covered by about 3 cm soil, 1-2 days before flooding. After the initial flood, around mid May, plots were hand sown at 180 kg/ha seed (rice variety Senia), pre-soaked in tapwater. Grain and straw yield and N content in plants were determined at maturity.Soil cyanobacterial population was measured from cores collected from each plot, at 4-5 weekly intervals throughout the growth cycle. Four in situ measurements of nitrogenase activity were made in May, June, July, and September of each year.

Reliability: (2) valid with restrictions

incomplete documentation of soil and of treatment regime

Flag: Critical study for SIDS endpoint

11-MAY-2003 (84)

Type: field
Species: other bacteria: nitrogen-fixing legume bacteria and nitrogen-fixing blue green algae
Unit: **Analytical monitoring:** yes

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate as fertiliser, no further details

Remark: The result is attributed to the low pH (4.4) of the soil, which results from ammonium sulfate addition. Toxic metal ions (e.g. Al) released from soil at acid pH may contribute to the effect. Soil pH can be increased by liming.
Result: Biological nitrogen-fixing activity was reduced (nitrogen-fixing legume bacteria) or eliminated (nitrogen-fixing blue green algae) in soil fertilised with ammonium sulfate.
Test condition: exposure period: more than 30 years; 80 kg N/ha applied annually.
The test site is located 60 degrees N, 17degrees E, near Uppsala in Central Sweden. The soil is post glacial clay (35% clay, 35% silt, 21% fine sand). Several organic and

mineral fertilisers are compared in this experiment, which began in 1956. All mineral plots were fertilised at 80 kg N /ha, and P and K were added to achieve equal rates of application to all fertilised plots. The 2m x 2m plots are dug to a 15 cm depth annually. The experiments reported here were carried out between 1988 and 1990. The crop rotation consisted of arable crops only, mainly cereals and oilseed crops, but also some root crops.
Nitrogen fixation was measured by acetylene reduction activity.

Reliability: (2) valid with restrictions
incomplete documentationof soil and treatment regime

Flag: Critical study for SIDS endpoint

25-APR-2003 (85)

Type: field
Species: other bacteria: total soil microbial bacteria
Unit: **Analytical monitoring:** yes

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate as fertiliser, no further details

Remark: Although the ammonium sulfate treated plots showed long term soil biomass reduction, soil respiration was not affected.

Result: The soil biomass (determined from ATP content) was reduced considerably, relative to the fallow and other fertilised plots. However, this effect is attributed to the low pH (4.4) of the soil, which results from ammonium sulfate addition. Soil pH can be increased by liming.

Test condition: exposure period: more than 30 years; 80 kg N/ha applied annually.
The test site is located 60 degrees N, 17degrees E, near Uppsala in Central Sweden. The soil is post glacial clay (35% clay, 35% silt, 21% fine sand). Several organic and mineral fertilisers are compared in this experiment, which began in 1956. All mineral plots were fertilised at 80 kg N /ha, and P and K were added to achieve equal rates of application to all fertilised plots. The 2m x 2m plots are dug to a 15 cm depth annually. The experiments reported here were carried out between 1988 and 1990. The crop rotation consisted of arable crops only, mainly cereals and oilseed crops, but also some root crops.Microbial biomass was determined from the soil ATP content. Prior to biomass determination, all soil samples were incubated for 2-4 weeks at 25 degrees C and 60-70% humidity.

Reliability: (2) valid with restrictions
incomplete documentation of soil and treatment regime

Flag: Critical study for SIDS endpoint

25-APR-2003 (86)

Species: aerobic microorganisms

Result: The results suggested that ammonium sulfate sensitive strains had a growth advantage in lower ammonium sulfate concentrations, while insensitive strains had a growth

advantage at higher ammonium sulfate concentrations. Both sensitive and insensitive strains were found in the primary and nightsoil sludges, with the sensitive strains predominating. This explained the operational observations in several sewage treatment plants concerning the efficacy of nitrifying bacteria.

Test condition: Ammonium sulfate toxicity information is available for both sewage treatment microorganisms and for microorganisms found in soil. The sewage treatment study investigated 14 strains of Nitrobacter spp. (ammonium oxidising bacteria) isolated from 25 different sludges including three sludges from primary sewage treatment plants and two sludges from nightsoil treatment plants. Nitrite production kinetic studies showed that insensitive strains (those which grew well at 4700 mg/lL ammonium sulfate) showed Monod growth, while sensitive strains (those which grew at 94 mg/lL but not at 4700 mg/lL) followed Haldane kinetics.

Reliability: (2) valid with restrictions

16-JUL-2004 (87)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

Species: other: Clarias batrachus
Endpoint: other: endocrine changes
Exposure period: 12 month
Unit: mg/l **Analytical monitoring:**
LOEC: = 100

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate fertilizer, not further specified

Remark: In this study the effect of ammonium sulfate on the histology and histochemistry of the thyrotropes, lactotropes, corticotropes, and the target organs thyroid and adrenal were studied. The results were compared with those seen in metopirone and thiourea treated animals and also after radiothyroidectomy.

Result: Prolonged exposure of Clarias batrachus to sublethal doses of ammonium sulfate fertilizer induced marked hypertrophy of the cortical and medullary cells of the interrenal gland and inhibition of thyroid function evident from the follicular hypertrophy, hyperplasia, and reduced colloid content with correlative histological changes in the pituitary corticotrophs and thyrotrophs of the experimentals.

The thyrotrophes of the control fish were small, and their nuclei and nucleoli were less prominent.

Results were similar in ammonium sulfate and thiourea treated fish.

Test condition: More than 60 Clarias batrachus were bought from the fish market at Varanasi, and acclimated to the laboratory conditions for ten days prior to the start of the experiments.
The fish were divided into three groups of ten each. One

group was given one mC of radioiodine I-131 in four equal instalments of 250 uCi at three monthly intervals for one year.
Another group was kept in 0.01 % ammonium sulfate for one year. Water was changed three times a week.
The third group was kept in 0.03 % thiourea for three months. The water was changed three times a week.
All fish were sacrificed by decapitation, and the adrenal and thyroid were fixed in Bouin`s sublimate. As the thyroid is in the form of scattered follicles in the region of the ventral aorta and its afferent branchial arches, the entire pharyngeal region was fixed. The picric acid of the fixative was found to be sufficient to decalcify the pharyngeal bones within a week. Paraffin sections were cut 3 and 5 um thick. The thyroid and adrenal were stained in periodic acid Schiff reagent (PAS)-haematoxylin and haematoxylin-eosin. The pituary was stained in PAS-lead haematoxylin (PbH).
All experiments were run with equal numbers of controls.

Reliability: (2) valid with restrictions
basic data are given

Flag: Critical study for SIDS endpoint

26-JAN-2004 (88)

Species: other: Channa punctatus
Endpoint: other: ovarian changes
Exposure period: 6 month
Unit: mg/l **Analytical monitoring:** no
LOEC: = 100

Method: other: see Test Condition
GLP: no
Test substance: other TS: ammonium sulfate fertilizer, not further specified

Remark: Maturation of oocytes through stages I,II and III was inhibited in the exposed fish. In stage III oocytes, the nucleus exhibited degenerative changes.

Result: No external manifestation of toxicity was observed, but ovarian growth was significantly reduced (gonadosomatic index of 1.13 vs 1.80 for the control).

Test condition: Ten adult C. punctatus purchased from a local fish market were exposed in a 40-liter aquarium to 100 ppm ammonium sulfate for six months. Ten additional fish were placed in a second aquarium and served as a control. Dilution water was well water with pH 7.2. Aquaria were maintained under natural light and temperature such that the average monthly water temperature from January to June was 20, 23, 27, 31, 32 and 35 °C, respectively. The water was changed every alternate day after feeding the fish with goat liver. All fish were sacrificed at the end of 6 months and the histology of the ovaries was examined.
The experiments were ran from January when the ovary was in the resting phase, and ended the last week of June when control fish exhibited spawning phase ovaries with matured vitellogenic stage-IV oocytes.

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint

26-JAN-2004 (89)

Species: other: Channa punctatus
Endpoint: other: testicular development
Exposure period: 6 month
Unit: mg/l **Analytical monitoring:** no
LOEC: = 100

Method: other: see Test Condition
GLP: no
Test substance: other TS: ammonium sulfate fertilizer, not further specified

Result: In testes of the 100 ppm group, spermatogenesis did not progress beyond spermatocyte stage and sperm were totally absent. Necrosis of spermatogenic elements and other effects were also noted. In the 500 ppm group, testes exhibiteddisorganization of lobules, significant inhibiton of spermatogenesis, extensive necrosis, and disintegration of spermatogenic elements. The gonado-systemic indices (=total gonad weight : total body weight x 100) were significnatly reduced in the 100 ppm (0.125) and 500 ppm (0.117) treated groups relative to the ocntrol (0.166).

Correlative histological changes were also observed in the pituitary gonadotrophs, in fish of both experimental groups, being smaller, involuted, inactive and less in number. The authors concluded that ammonium sulfate is inhibiting testicular development and inducing deleterious changes in spermatogenic elements either by direct action on the testis itsself or indirectly via the hypothalamic-pituitary- testicular axis in this species.

Test condition: Thirty adult C. punctatus were divided between 100 ppm, 500 ppm and control aquariums in well water at pH 7.2. Water changes were made on alternate days after feeding fish with minced goat liver.
The experiments were run from the first week in January (when testes were in resting condition containing only spermatogonia), and ended the last week of June (when control testes were in mature spawning conditions).

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
26-JAN-2004 (90)

Species: Oncorhynchus gorbuscha (Fish, fresh water)
Endpoint: other: length of fish, weight of fish
Unit: **Analytical monitoring:** yes

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Result: NOEC (61 d) = 1.2 mg un-ionized NH3/L (length, weight of fry).

LOEC (61 d) = 2.4 mg un-ionized NH3/L (length, weight of fry). The highest concentration of ammonia caused significant decreases in weight of exposed fry in all three exposure groups. At 2.4 ppb un-ionized ammonia, the groups held for 40 and 61 days were significntly smaller in length and weight but at 1.2 ppb un-ionized ammonia there was no significant difference. Effects were consistently more adverse for groups held 61 days.

Test condition: Three groups of alevins were exposed to ammonium sulfate solutions for three different lengths of time (21, 40, or 61 days).
In each group, subgroups were exposed to concentrations of unionized ammonia ranging from 0 to 4 ppb. Test organisms were exposed at pH 6.3 - 6.5 and 3.7 - 4.8 °C.
Reliability: (2) valid with restrictions
limited documentation
22-JUL-2004 (28)

Species: other: Channa punctatus
Endpoint: other: ovarian changes
Exposure period: 6 month
Unit: mg/l **Analytical monitoring:** no
LOEC: = 500

Method: other: see Test Condition
GLP: no
Test substance: other TS: ammonium sulfate fertilizer, not further specified

Remark: In comparison to the untreated control, the ovarian growth was significantly reduced. The gonadosomatic index (total ovary weight : total body weight x 100) was reduced and oocytes of ammonium sulfate treated fish were mostly at stage I of oocyte development and no mature oocytes (stage IV) occured. Histological examination revealed a number of oocytes with proteinaceous extra- and intranuclear "inclusion bodies". These oocytes degenerated. The author concluded that ammonium sulfate is affecting ovarian growth, however no mode of action could be deduced from this study.
Result: No external manifestation of toxicity was observed, but ovarian growth was significantly reduced (gonadosomatic index of 1.18 vs 1.92 for the controls).
Test condition: Forty adult C. punctatus were caught in the wild, acclimated to laboratory conditions and split up between two 30-liter glass aquaria containing water at pH 7.2. One group was exposed to 500 ppm ammonium sulfate, and the other group served as the control. The experiment ran from the first week of January when the ovary was in the resting phase, and ended the last week of June when control fish exhibited spawning phase ovaries.
Reliability: (2) valid with restrictions
10-MAR-2003 (91)

Species: other: Channa punctatus
Exposure period: 6 month

Remark: A dose-dependend effect on liver and thyroid has was reported when adult fish were exposed to 100 ppm ("safe concentration") or 500 ppm ("sublethal concentration") of ammonium sulfate for 6 month. Hepatocytes revealed initial hypertrophy followed by exhaustion as evidenced by de-granulation, nuclear pyknosis and focal necrosis. Thyroid follicles exhibited various degrees of hypertrophy, hyperplasia, hyperemia and reduction in colloid content. The authors concluded that ammonium sulfate is causing

a dysfunction in the liver and thyroid causing alterations in the physiology of the fish, however no mode of toxicity could be deduced.
The effect on the adrenals was also examined after exposure to 100 and 500 ppm for 6 months. Fish exposed to 500 ppm appeared stressed, but no mortality occurred. The cortical (corticosteroid producing) cells of the adrenals in both treated groups showed extensive hyperplasia, degranulation, involution and exhaustion. The histopathological findings showed a slight hypertrophy at 100 ppm whereas at 500 ppm atrophic changes occurred.

Reliability: (2) valid with restrictions
basic data are given

26-JAN-2004 (92)

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

Species: other terrestrial plant: onion (allium cepa L. - Norstar 210B)
Endpoint: growth
Expos. period: 84 day(s)

Method: other: see Test Condition
Year: 1995
GLP: no data

Result: Yield (dry weight per pot) was greatest for 133 mg N per kg soil. An inhibitory effect was found at 399 and 532 mg N per kg soil, except for the sandy soil where only the 532 mg N per kg soil ammonium sulfate was inhibitory.
Test condition: The effects of several nitrogen compounds on the growth of onion was studied in 4 Canadian soils (pH range 5.0 to 6.6, organic matter range 6-41.5 g/kg, sand or silty or sandy loams, podzols or gleysols). Hydrated lime was added to the lower pH soils to raise the pH to near 6.5, and P, K and Mg were added to all soils to prevent deficiencies. After seiving, air drying and moisture adjustment to 80% water holding capacity, the soils were placed in 3.2 l plastic pots, into which four 14 day old onion seedlings were transplanted. Ammomium sulfate was added at 0, 133, 266, 399, and 532 mg N per kg soil. The growth chamber was maintained at 22-18C and a 16-8 h day/night cycle, 65% relative humidity, and 300µE m-2 s-1 photon flux density at plant height. Immature plants were harvested 84 days after transplanting. Bulbs, roots, and leaves were separated, and fresh and dry weights of all plant parts were determined.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
22-JUL-2004 (93)

Species: other terrestrial plant: Avena sterilis spp Macrocarpa Mo.
Expos. period: 21 day(s)
Unit: mg/l

Method: other: see Test Condition
Year: 1989
GLP: no data
Test substance: other TS: ammonium sulfate, no further details

Remark: ppm is specified in the test, not ppm N. Note no control of pH or ionic strength
Result: No significant increase in germination rate compared with the control. At 5000 ppm, an inhibitory effect was found.
Test condition: The effects of several nitrogen compounds on the germination of the first seed of each spikelet of Avena sterilis spp Macrocarpa Mo. was studied at 15 C, or at alternating temperatures of 15 C (16 hours) and 5 C (8 hours). 100 seeds taken at random were placed in a covered Petrie dish, wrapped in filter paper soaked in 20 ml of the test solution. Concentrations studied are 0, 100, 1000, 2500, and

5000 ppm ammonium sulfate. Germination was checked every 3 days, until conclusion of the test due to lack of further germination on day 21.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
26-JAN-2004 (94)

Species: other terrestrial plant: Picea abies
Expos. period: 60 month

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, solid

Result: Ammonium sulfate application of 100 kg ha-1 yr-1 N and 114 kg ha-1 yr-1 S spread as solid ammonium sulfate from 1988 to 1993 made the trees more susceptible to drought, compared to those treated with N-free fertiliser. This could be seen as a reduction in the flushing of new shoots.

Test condition: TEST ORGANISMS: Picea abies L. Karst, planted in 1966 on a clear cut site. The stand had an average basal area of about 25 m2 ha-1 when the 45m x 45m field plots were established in 1987.TEST SITE: Located in S. W. Sweden (lat. 560 33', long. 130 13') about 16 km from the coast and 95-115 m above sea level. The mean annual precipitation is about 1100 mm. May and June are often very dry. The annual mean air temperature is about 7.50C. The mean pH of precipitation is 4.5 (1988-1991), and the deposition adds 20-25 kg ha-1 yr-1 N and 10-55 kg ha-1 yr-1 S. The soil is a Haplic podzol on a loamy sand till, with a pHH2O of ca.3.9 in the humus layer and between 4.0 and 4.6 in the mineral soil down to 50 cm depth.
TEST DESCRIPTION: 100 kg ha-1 yr-1 N and 114 kg ha-1 yr-1 S were spread as solid ammonium sulfate from 1988 to 1993. The experiment was set up in a randomised block desigh with 4 replicates, except for the drought experiment, which was without replication. In the drought experiment, roofs were places 1-1.5 m above ground, preventing 2/3 of the throughfall from reaching the ground, during April - September 1992-1993.Needles were collected from dominant or co-dominant trees, with diameters equal to the mean diameter and to the mean + 1 standard deviation.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
26-JAN-2004 (95)

Species: other terrestrial plant: Phaseolus vulgaris L.
Expos. period: 13 day(s)

Remark: 1) The authors suggest that, for the plant development stage studied, the changes seen were not signifigantly related to plant growth and development.
2) The ammonium sulfate application rate is stated to be about 2 orders of magnitude above ambient episode concentration.

Result: Plants exposed to 26 mg/m3 ammonium sulfate aerosol for up to 320 hours showed no changes in plant biomass or leaf area. However, visible foliar injury occurred, and both

abaxial and adaxial leaf resistances were decreased from control values.

Test condition: TEST ORGANISMS: Pinto bean plants (Phaseolus vulgaris L.) 14 days in age at the beginning of the test. All plants were staked to ensure an erect growth habit.
TEST VESSEL: environmental growth chamber, Daytime climate 45-55% r.h. 250C. Night-time climate 70-80% r.h. 200C.

Flag: Critical study for SIDS endpoint

26-JAN-2004 (26)

Species: other terrestrial plant: Orobanche crenata Forsk
Expos. period: 7 day(s)
Unit: mmol/l

Method: other: laboratory test, see Test Conditions
GLP: no data
Test substance: other TS: ammonium sulfate, analytical grade reagent, no further details

Remark: Orobanche crenata Forsk. is a parasite weed.
Result: 4 mM ammonium sulfate reduced the germination % of O. Crenata from about 50% in the controls to 16%. In the presence of the nitrification inhibitor nitrapyrin, the germination percentage of O. Crenata was reduced from c.55% in the controls to 2%.

Test condition: TEST ORGANISMS: Orobanche crenata Forsk. seeds were collected in Syria, at the Tel Hadya research station of the Center for Research in Dry Areas (3600'N, 36056'E), and stored in the dark at room temperature (15-250C) until use, 5-6 years later. The seeds were then surface-sterilised in sodium hypochlorite solution (1% chlorine, wt/V) for 5 minutes, and then thoroughly rinsed with distilled water. This treatment prevented fungal contamination to a large extent.
TEST VESSEL: Glassware, including 5 cm Petri dishes, and filters used in the experiment were sterile. Distilled water was used to prepare the solutions.
TEST METHODOLOGY: 25-30 seeds chosen at random were evenly spread on a 1 cm diameter Whatman GF/C glass fibre filter. Three of these 1 cm filters with seeds were placed on a 4.7 cm diameter GF/C filter located in a 5 cm diameter Petri dish, to which was added 2 ml of 0.3 mM pH 7 Hepes buffer [N-(hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid)]. There were 4 replicate Petri dishes for each concentration used in this experiment. For conditioning, the Petri dishes were then sealed, wrapped individually in aluminium foil, and kept at 20 + 1 0C for 14 days. After conditioning, the GF/C filters with seeds were taken out of the Petri dishes and briefly (c. 30 seconds) allowed to dry. The filters with seeds were then transferred to another Petri dish, to which 2 ml of a stimulating solution was added. This solution contained 1 mg/l of the synthetic germination stimulant GR24 (see original paper for details) and 0.1% v/v acetone in the different test solutions (including water alone, Hepes buffer alone, buffer + 8 mg/l nitrapyrin, 4 mM (NH4)2S04, and 4 mM (NH4)2S04 + nitrapyrin. At 7 days after the addition of the stimulating solution, the germination percentages (and also the lengths of the germ tubes) were

determined.
Reliability: (2) valid with restrictions
Concentrations not measured. Limited documentation of results.
11-MAY-2003 (96)

4.6.3 Toxicity to Soil Dwelling Organisms

Type: other: field
Species: other: Collembola
Endpoint: other: number of collembola

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, 150 kg N/ha/a

Remark: This test simulates ammonium sulfate deposition by acid rain. Fertiliser application levels could be higher.
Result: In the first year evaluation, a significant increase of the numbers of Cryptostigmata in the organic layer to more than double of control level was detected. In the second year, the abundances of Cryptostigmata were still high, but the differences to control were not significant.
As the concentrations applied were in excess of those delivered by acid rain, it was concluded that acid rain would not have a detrimental effect on soil collembola.
Test condition: Simulated rainfall delivered 150 kg N ha -1 a -1 (708 kg (NH4)2SO4 ha -1 a -1) in monthly increments to a field planted with Picea abies at 2500 ha-1, with no ground vegetation. The trees were approximately 14 m high at the time of the experiment, and the canopy covered approx. 90% of the test area. The salt was dissolved in 1 l of water, and sprayed on 6m2 plots in a randomised block design. The soil pH of both experimental and control plots was 5.3 at the end of the test. The soil fauna were extracted and counted twice, at the end of year 1 and at the end of year 2.
Reliability: (2) valid with restrictions
concentration not measured. Only one concentration studied
Flag: Critical study for SIDS endpoint
22-JUL-2004 (97)

Type: other: field
Species: other: Cryptostigmata (family Acarina)
Endpoint: other: number of cryptostigmata

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, 150 kg N/ha/a

Remark: This test simulates ammonium sulfate deposition by acid rain. Fertilizer application levels could be higher. Similar results were also found for other Acari, i.e. Prostigmata and Astigmata (mineral layer only) at the end of year 1, and Astigmata in both layers at the end of year 2. However, Mesostigmata numbers did not differ significantly from control in either year. No significant decrease was found in

the numbers of any of the acari studied.
Result: In the first year evaluation, a significant increase of the numbers of Cryptostigmata in the organic layer to more than double of control level was detected. In the second year, the abundances of Cryptostigmata were still high, but the differences to control were not significant.
As the concentrations applied were in excess of those delivered by acid rain, it was concluded that acid rain would not have a detrimental effect on soil collembola.
Test condition: Simulated rainfall delivered 150 kg N ha -1 a -1 (708 kg (NH4)2SO4 ha -1 a -1) in monthly increments to a field planted with Picea abies at 2500 ha-1, with no ground vegetation. The trees were approximately 14 m high at the time of the experiment, and the canopy covered approx. 90% of the test area. The salt was dissolved in 1 l of water, and sprayed on 6m2 plots in a randomised block design. The soil pH of both experimental and control plots was 5.3 at the end of the test. The soil fauna were extracted and counted twice, at the end of year 1 and at the end of year 2.
Year: 1989-1991.
Exposure Period: 2 years.
Reliability: (2) valid with restrictions
Concentration not measured. Only one concentration studied.
Flag: Critical study for SIDS endpoint
22-JUL-2004 (97)

Species: other: Ciliata
Exposure period: 120 day(s)
Unit: other: kg/ha

Method: other: see Test Condition
Year: 1983
GLP: no data
Test substance: other TS: ammonium sulfate, no further details

Result: A LC50 value could not be determined because after 4 months the abundance had increased by 2-3 times that in the control plot for all dose levels. No further details reported. However, the results indicate that ammonium sulfate is not toxic to ciliata in alpine pasture.
Test condition: Three sites of alpine pasture were treated with 100, 400, and 1200 kg/ha ammonium sulfate on 13 June. Nematodes, Testacea, and Ciliates were collected from each site and from the control site on July 5, Sept 5, and Oct 18. Ciliata biomass relative to controls was independent of ammonium sulfate concentration, but after 4 months the abundance at the treated sites were 2-3 times higher than at the control plot.
Reliability: (4) not assignable
Abstract of conference presentation. Limited documentation. Pilot study.
25-APR-2003 (98)

Species: other: Meloidogyne incognita (root-knot nematode)
Exposure period: 72 hour(s)
Unit: mg/kg soil dw

Method: other: see Test Condition
Year: 1984
GLP: no data
Test substance: other TS: ammonium sulfate, no further details

Remark: (1) - Other tests in the paper look at recovery of nematodes when ammonium sulfate is removed, and at the effects of ammonium sulfate on the tomato plants.
(2) - Neither ionic strength nor pH are controlled in this experiment. Both will differ between the solutions used.
Result: After 72 hours, 95% of the nematodes in the 9900 ppm N solution had died. Approximately 50% of the nematodes in the 1100 ppm N solution had died after 72 hours.
Test condition: Nematodes were placed in 1100, 3300, or 9900 ppm N solutions of ammonium sulfate in distilled water for 24, 48, and 72 hours.
Reliability: (4) not assignable
limited documentation of results
25-APR-2003 (99)

Species: other: Nematoda
Exposure period: 120 day(s)
Unit: other: kg/ha

Method: other: see Test Condition
Year: 1983
GLP: no data
Test substance: other TS: ammonium sulfate, no furrther details

Result: A LC50 value could not be determined because after 4 months the number of nematodes had increased by a factor of 3. No further details reported. However, the results indicate that ammonium sulfate is not toxic to Nematoda in alpine pasture.
Test condition: Three sites of alpine pasture were treated with 100, 400, and 1200 kg/ha ammonium sulfate on 13 June. Nematodes, Testacea, and Ciliates were collected from each site and from the control site on July 5, Sept 5, and Oct 18. Nematode numbers dcreased on July 5, but by 18 October 3 times as many nematodes were collected from the fertilised sites as from the control site.
Reliability: (4) not assignable
Abstract of conference presentation. Limited documentation. Pilot study.
25-APR-2003 (98)

Species: other: Testacea
Exposure period: 120 day(s)
Unit: other: kg/ha

Method: other: see Test Condition
Year: 1983
GLP: no data
Test substance: other TS: ammonium sulfate, no further details

Result: A LC50 value could not be determined because the biomass grew more at the highest dose level (1200 kg.ha) than in the control. No further details reported. However, the results indicate that ammonium sulfate (AS) at 1200 kg/ha is not

toxic to Testacea in alpine pasture.
Test condition: Three sites of alpine pasture were treated with 100, 400, and 1200 kg/ha Ammonium sulfate on 13 June. Nematodes, Testacea, and Ciliates were collected from each site and from the control site on July 5, Sept 5, and Oct 18. Testacea biomass was unaffected at the lower ammoniumsulfate levels, but increased by a factor of 1.4 at the highest level.
Reliability: (4) not assignable
Abstract of conference presentation. Limited documentation. Pilot study.
25-APR-2003 (98)

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

Species: other: Pseudacris regilla (Pacific treefrog)
Expos. period: 10 day(s)

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, 99.2% pure (Reagent Grade, Mallinckrodt Baker Inc, Phillipsburg, NJ)

Remark: These tadpoles are from different egg masses than those used for the 9 week-old test (see following IUCLID entry).
Result: The NOEC for the tadpoles, which were 6 weeks old at the start of the 10 day test, was 17.4 mg/L ammonium nitrogen. The corresponding LOEC was 37.0 mg/L ammonium nitrogen.
Test condition: TEST ORGANISMS: Eggs of Pseudacris regilla were collected locally from non-agricultural areas and hatched in the EPA laboratory facilities at Corvallis, Ore. Tadpoles were subsequently reared in aquaria with a continuous flow of temperature controlled fresh water whose temperature was gradually increased from the collection temperature of ca. 10 deg C to the test temperature of 20 +/- 1 deg C. Temperature was continuously recorded. They were held at a photoperiod of 14:10 light:dark. Newly hatched Pseudacris regilla were fed pelleted rabit feed, with small amounts of newly hatched brine shrimp and then frozen fish food.
TEST VESSEL: The test species were exposed to ammonium sulfate in 18 10-L aquaria with a continuously-flowing water diluter system that automatically delivered six concentrations of chemical and controls to three aquaria per concentration.
PHYSICAL/CHEMICAL PARAMETERS: Rearing and test water was obtained from wells near the Willamette River at Corvallis, Oregon. Dissolved oxygen, measured by electrode, was maintained near saturation by the flowing water. pH (median value 7.3) was measured by electrode. Total hardness (72 +/- 4.2 mg/L), alkalinity (63 +/- 2.8 mg/L), and conductivity (188.8 +/- 7.3 µS/cm) were measured by EPA methods 130.2, 310.1, and 120.1, respectively. Background ammonium-N ranged from 0.005 - 0.010 mg/L in well water, and from 0.00 to 0.03 mg/L in control tanks. Water samples for measurement of ammonium sulfate were taken from each concentration (each aquarium in the diluter) on four days, and analysed with a Hach DR/700 digital photometer. Quality assurance techniques were used in the analysis.

TEST CONDITION: Five 6-week old tadpoles were used in each of three replicate aquaria, and the test was run for 10 days. Length and mean wet wet were measured at the end of the test. The means and standard deviations of the ammonium-nitrogen concentrations measured at the 5 experimental concentrations and in the control were 211.2 +/- 17.5, 118.0 +/- 9.8, 66.6 +/- 7.7, 37.0 +/- 4.6, 17.4 +/- 3.8 and 0 mg/L ammonium nitrogen, respectively. Ten day LOEC values and NOEC values were determined with the Dunnetts multiple comparison procedure.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
22-JUL-2004 (67)

Species: other: Pseudacris regilla (Pacific treefrog)
Expos. period: 10 day(s)

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, 99.2% pure (Reagent Grade, Mallinckrodt Baker Inc, Phillipsburg, NJ)

Remark: These tadpoles are from different egg masses than those used for the 6 week-old test, (previous entry).
Result: The NOEC for the tadpoles, which were 9 weeks old at the start of the 10 day test, was 32.4 mg/L ammonium nitrogen. The corresponding LOEC was 52.5 mg/L ammonium nitrogen.
Test condition: TEST ORGANISMS: Eggs of Pseudacris regilla were collected locally from non-agricultural areas and hatched in the EPA laboratory facilities at Corvallis, Ore. Tadpoles were subsequently reared in aquaria with a continuous flow of temperature controlled fresh water whose temperature was gradually increased from the collection temperature of ca. 10 0C to the test temperature of 20 + 1 deg C. Temperature was continuously recorded. They were held at a photoperiod of 14:10 light:dark. Newly hatched Pseudacris regilla were fed pelleted rabit feed, with small amounts of newly hatched brine shrimp and then frozen fish food.
TEST VESSEL: The test species were exposed to ammonium sulfate in 18 10-L aquaria with a continuously-flowing water diluter system that automatically delivered six concentrations of chemical and controls to three aquaria per concentration.
PHYSICAL/CHEMICAL PARAMETERS: Rearing and test water was obtained from wells near the Willamette River at Corvallis, Oregon. Dissolved oxygen, measured by electrode, was maintained near saturation by the flowing water. pH (median value 7.2) was measured by electrode. Total hardness (34 +/- 0 mg/L), alkalinity (34 +/- 0 mg/L), and conductivity (104.4 +/- 1.2 µS/cm) were measured by EPA methods 130.2, 310.1, and 120.1, respectively. Background ammonium-N ranged from 0.005 - 0.010 mg/L in well water, and from 0.00 to 0.03 mg/L in control tanks. Water samples for measurement of ammonium sulfate were taken from each concentration (each aquarium in the diluter) on two days, and analysed with a Hach DR/700 digital photometer. Quality assurance techniques were used in the analysis. TEST CONDITION: Five 6-week old tadpoles were used in each of three replicate aquaria, and the test was run for 10 days. Length and mean wet wet were measured

at the end of the test. The means and standard deviations of the ammonium-nitrogen concentrations measured at the 5 experimental concentrations and in the control were 211.5 + 16.3, 126.5 + 4.9, 81.5 + 7.8, 52.5 + 4.9, 32.4+ 3.3 and 0 mg/l ammonium nitrogen, respectively. Ten day LOEC values and NOEC values were determined with the Dunnetts multiple comparison procedure.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
22-JUL-2004 (67)

Species: other: Ambystoma gracile (Northwestern salamander)
Expos. period: 10 day(s)

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, 99.2% pure (Reagent Grade, Mallinckrodt Baker Inc, Phillipsburg, NJ)

Result: The NOEC for the larvae, which were 5 weeks old at the start of the 10 day test, was 81.5 mg/L ammonium nitrogen. The corresponding LOEC was 126.5 mg/L ammonium nitrogen.

Test condition: TEST ORGANISMS: Eggs of Ambystoma gracile were collected locally from non-agricultural areas and hatched in the EPA laboratory facilities at Corvallis, Ore. They were subsequently reared in aquaria with a continuous flow of temperature controlled fresh water whose temperature was gradually increased from the collection temperature of ca. 10 deg C to the test temperature of 20 +/- 1 deg C. Temperature was continuously recorded. They were held at a photoperiod of 14:10 light:dark. Newly hatched Ambystoma gracile were fed newly hatched brine shrimp, and then daphnids (D. pulex and D. magna) and annelid worms (Lumbriculus variegatus) as they grew larger.
TEST VESSEL: The test species were exposed to ammonium sulfate in 18 10-L aquaria with a continuously-flowing water diluter system that automatically delivered six concentrations of chemical and controls to three aquaria per concentration.
PHYSICAL/CHEMICAL PARAMETERS: Rearing and test water was obtained from wells near the Willamette River at Corvallis, Oregon. Dissolved oxygen, measured by electrode, was maintained near saturation by the flowing water. pH (median value 7.2) was measured by electrode. Total hardness (34 +/- 0 mg/L), alkalinity (34 +/- 0 mg/L), and conductivity (104.4 +/- 1.2 µS/cm) were measured by EPA methods 130.2, 310.1, and 120.1, respectively. Background ammonium-N ranged from 0.005 - 0.010 mg/L in well water, and from 0.00 to 0.03 mg/L in control tanks. Water samples for measurement of ammonium sulfate were taken from each concentration (each aquarium in the diluter) on two days, and analysed with a Hach DR/700 digital photometer. Quality assurance techniques were used in the analysis.
TEST CONDITION: Four 5-week old larvae were used in each of three replicate aquaria, and the test was run for 10 days. Length and mean wet wet were measured at the end of the test. The means and standard deviations of the ammonium-nitrogen concentrations measured at the 5 experimental concentrations and in the control were 211.5

+/- 16, 126.5 +/- 5, 81.5 +/- 5, 52.5 +/- 5, 32.4 +/- 3, and 0 mg/L ammonium nitrogen, respectively. Ten day LOEC values and NOEC values were determined with the Dunnetts multiple comparison procedure.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
22-JUL-2004 (67)

Species: other: Rana aurora (Redlegged frog)
Expos. period: 10 day(s)

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, 99.2% pure (Reagent Grade, Mallinckrodt Baker Inc, Phillipsburg, NJ)

Result: The NOEC for the tadpoles, which were 4 weeks old at the start of the 10 day test, was 82.7 mg/L ammonium nitrogen. The corresponding LOEC was 134.0 mg/L ammonium nitrogen.

Test condition: TEST ORGANISMS: Eggs of Rana aurora were collected locally from non-agricultural areas and hatched in the EPA laboratory facilities at Corvallis, Ore. Tadpoles were subsequently reared in aquaria with a continuous flow of temperature controlled fresh water whose temperature was gradually increased from the collection temperature of ca. 10 0C to the test temperature of 20 +/- 1 deg C. Temperature was continuously recorded. They were held at a photoperiod of 14:10 light:dark. Newly hatched Rana aurora were fed pelleted rabit feed, with small amounts of newly hatched brine shrimp and then frozen fish food.
TEST VESSEL: The test species were exposed to ammonium sulfate in 18 10-L aquaria with a continuously-flowing water diluter system that automatically delivered six concentrations of chemical and controls to three aquaria per concentration.
PHYSICAL/CHEMICAL PARAMETERS: Rearing and test water was obtained from wells near the Willamette River at Corvallis, Oregon. Dissolved oxygen, measured by electrode, was maintained near saturation by the flowing water. pH (median value 7.2) was measured by electrode. Total hardness (28.5 +/- 7.8 mg/L), alkalinity (30.0 +/- 7.1 mg/l), and conductivity (87.0 +/- 17.8 µS/cm) were measured by EPA methods 130.2, 310.1, and 120.1, respectively. Background ammonium-N ranged from 0.005 - 0.010 mg/l in well water, and from 0.00 to 0.03 mg/L in control tanks. Water samples for measurement of ammonium sulfate were taken from each concentration (each aquarium in the diluter) on two days, and analysed with a Hach DR/700 digital photometer. Quality assurance techniques were used in the analysis.

TEST CONDITION: Five 4-week old tadpoles were used in each of three * replicate aquaria, and the test was run for 10 days. Length and mean wet wet were measured at the end of the test. The means and standard deviations of the ammonium-nitrogen concentrations measured at the 5 experimental concentrations and in the control were 227.0 +/- 1.4, 134.0 +/- 7.1, 82.7 +/- 3.2, 50.5 +/- 4.9, 28.8 +/- 5.2 and 0 mg/L ammonium nitrogen, respectively. Ten day

LOEC values and NOEC values were determined with the Dunnetts multiple comparison procedure.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
22-JUL-2004 (67)

Species: other: hen
Endpoint: weight
Expos. period: 14 day(s)
Unit: ppm
LOEC : ca. 1000

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Result: A reduction in weight gain and gain/ feed ratio with ammonium sulfate was noted when the concentration in the food exceeded 1 g/100 g food (10.000 mg/kg food). This effect was also observed with potassium sulfate while sodium-, magnesium- and calcium sulfate had little or no effect on the parameters determined in this study. No substance-related lethality occurred.
Test condition: The effect on weight gain and gain/feed ratio was examined in 14 day old chicks fed with different sulfate salts including ammonium sulfate. Day-old, male chicks of two strains (strains 5 and 10, Crawford) were provided with basal diet and water ad libitum for 14 days. The chicks were then individually weighted and divided into groups of 10. During the 11-day experimental period the birds were fed ad libitum and had free access to fresh water. Group weights were recorded at the beginning and end of the experiment. Feed consumption was measured on a group basis. In a second experiment two strains of female broiler chicks were given 24 diets. Twelve diets consisted of a chick starter diet supplemented with potassium or ammonium sulfate at levels calculated to supply 0, 1, 2, 3, 4, or 5 g of sulfate per 100 g of basal diet. the other 12 diets contained potassium or ammonium carbonate at levels calculated to provide the same levels of the cations as were added to the 12 sulfate diets. The experimental period in the second experiment was 14 days. The data for weight gain and gain:feed ratio were subjected to analysis of variance.
Reliability: (4) not assignable
Documentation insufficient for assessment.

22-MAY-2003 (100)

Species: other: Thermonactus basillaris (Harris) (beetle)
Expos. period: 24 hour(s)
Unit: other: kg active ingredient / ha

Method: other: rice field - aerial spraying, see also Test conditions
GLP: no data
Test substance: other TS: ammonium sulfate, no further details

Remark: Thermonactus basillaris (Harris), a predacious diving beetle, is economically important in that it is a predator of mosquito larvae.

Result: No beetle mortality was found upon application of 23.52 kg Al ammonium sulfate per ha. Application of 35.29 kg ammonium sulfate / ha resulted in 4.4% beetle mortality, 24 hours after application.

Test condition: TEST ORGANISMS: Adult Thermonactus basillaris (Harris) were collected in CDC minature light traps located at the Rice Research station near Crowley, Louisiana, approximately 24 hours prior to the field test. The beetles were maintained in a 45.5 l container partially filled with well water before being transported to the test site. Culex quinquefasciatus Say larvae from a laboratory colony were provided as a food source, to prevent canabalism among the beetles.
TEST VESSEL: For each test, five styrofoam floats (39.37x26.67x2.54 cm) were placed throughout a previously selected rice field. Each float contained 2 holes, into each of which a 500 ml beaker was inserted. Each beaker contained 400 ml of well water and five Thermonactus basillaris (Harris) adults. The beakers were covered with a 1.27 cm hardwire mesh, to prevent beetle escape. Two additional beakers with 5 adult beetles in each served as controls for each test.
TEST METHODOLOGY: Aerial application of the chemicals was made using a Grunman AgCat aircraft. Each agrichemical was applied according to rates and specifications printed on the label. Approximately 1 hr after the agriculture application, the beakers were removed from the site by suitably protected personnel, and beetle mortalities were recorded. The beakers were then transported to the laboratory, and posttreatment mortalities after 6, 12, and 24 hours were determined.

Reliability: (2) valid with restrictions
Concentrations not measured. Limited documentation of results. Limited description of rice field parameters.

11-MAY-2003 (101)

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

Memo: Comment on ammonium sulfate effect on mycorrhizal funghi

Remark: Effects of ammonium sulfate, deposited both as a fertiliser and as acid rain, on mycorrhizal funghi, have recently been summarised (ref). All results are dependent upon the vegetation and soil type. With regard to fertiliser deposition, several reports indicate a decrease in colonisation by ectomycorrhizal fungi (ECM), though this effect may be short lived, with 2 studies showing recovery 3 years and 13 years after fertiliser deposition. Several other reports show no decline in ECM colonisation. Reports of both types are available for pine forests, upland northern hemisphere sites, and heathland. The majority of reports do indicate some shift in the structure of the ECM community.

Results from field investigations of the percentage of mycorrhizal colonisation in stands affected by acid rain deposition are equivocal, with some finding decreased ECM and AM (arbuscular mycorrhizal fungi) colonisation, and others showing no effect. However, short term glasshouse studies generally (but not always) show decreases in percentage ECM colonisation at pH values below about 3.5. Interestingly, a study at pH2.4 has shown increases ECM colonisation, presumably due to the fertilising effect of increased N input. In general, AM fungi colonisation is reduced by acid deposition, though the effect may be less obvious in heathland. Acidification also appears to have a differential effect on different AM fungi taxa.

Reliability: (4) not assignable

15-MAY-2003 (102)

Remark: The filter feeding fresh water mussel Lamellidens marginalis L was shown to reduce the ammonium content in a 7 day test at pH 7.5 using ammonium sulfate solutions ranging from 10 to 200 ppm ammonium sulfate, with reduction beginning on day 2 in 10 and 25 ppm solutions, day 3 in 50 ppm solutions, day 4 in 100 and 150 pm solutions, and day 5 in 200 ppm solutions. This is consistent with the overall biopurification ability shown by mussels.

Reliability: (4) not assignable
The description of the test and of the treatment of the controls is limited.

15-MAY-2003 (103)

5.0 Toxicokinetics, Metabolism and Distribution

In Vitro/in vivo: In vivo
Type: Excretion
Species: other: hamster, guinea pig, rabbit

Result: It was demonstrated that the particles with a size of 0.3 and 0.6 um (MMAD) reached the lung, however a substantial proportion of the compound was found in the nose and the GI tract. Total respiratory tract deposition was greater with the larger particle size in all studies. The clearance from the lung (via the blood and urinary tract) was determined to be T1/2 = 18 to 20 minutes, and rate of lung clearance was similar for the two particle sizes and for all species.
The T1/2 for blood was determined in hamster: 3 hours, guinea pig: 1-3 hours, rabbit: > 6 hours. Hamster showed a large initial GI deposition whereas for rabbits and guinea pigs the maximum was reached only 1 hour after exposure.
By six hours after exposure 96% of the total collectable sulfate was present in the urine. The results of clearance experiments performed in this study suggested that there was no specific difference. Apart from the hamsters, the recovery of 35S in the urine was incomplete.
Test condition: Hamsters (n=8/group) (whole body), guinea pigs (n=12/group)(whole body fur protected) and rabbits (whole body and nose only)(number not reported) were studied for deposition and clearance of inhaled ammonium sulfate (1 - 3 mg/m3; MMAD 0.3 and 0.6 um, 0.05% and 0.5%).
The studies were performed using S35-labeled ammonium sulfate aerosols with high specific activity. A five minute exposure time and a short, reproducible time period in which tissues (blood, lung, nose, urine, GI tract) were obtained for the first analysis after exposure were necessary to determine deposition. Clearance was then
measured at 1, 3 and 6 hours after exposures.
Conclusion: Sulfate from the highly water soluble ammonium sulfate was cleared very rapidly from the body.
Reliability: (2) valid with restrictions
limited documentation
Flag: Critical study for SIDS endpoint
02-DEC-2003 (104)

In Vitro/in vivo: In vivo
Type: Absorption
Species: rabbit
Vehicle: physiol. saline
Route of administration: gavage

Method: other: see Test condition
GLP: no data
Test substance: other TS: ammonium sulfate, not

Result: The concentration of ammonium ion in serum had already increased remarkably 5 min after ingestion (1095 ug/dl) until it reached 11000 ug/dl. Inorganic sulfate concentration

started to increase at 10 min after ingestion and its level continued to increase linearly to 20 mEq/l.

Test condition: Five Japanese white rabbits, weighing 3.4 to 3.8 kg were used. The animals were anesthetized with intravenous pentobarbital sodium (12.5 mg/kg bw), injected in auricular veins, and supplemental doses were intermittently given to maintain surgical level of anesthesia. Rabbits were fixed in supine position and a double channel catheter was placed in the femoral artery to draw blood samples. The total dose of 1500 mg/kg ammonium sulfate (dissolved in saline solution) was administered in 10-15 mL of volume per rabbit through gastric probe in three rabbits. 2 rabbits were administered 10-15 mL of saline and used as controls.
Blood samples were collected from the femoral artery 5 min before and 10 min after and every 15 minutes up to 90 minutes after administration of ammonium sulfate solution.
The serum level of ammonium ion was measured.
Inorganic sulfate ions in serum were measured by ion chromatography.

Reliability: (2) valid with restrictions
limited documentation

Flag: Critical study for SIDS endpoint

10-APR-2006 (105)

In Vitro/in vivo: In vitro
Type: Metabolism
Species: rat

Result: In vitro addition of ammonium chloride to rat liver slices produced an increase in urea synthesis of 38%. No additional increase in the production of urea was found in liver slices prepared from rats injected intraperitoneally with 10.6 mmol NH4-N/kg as ammonium sulfate and incubation of ammonium chloride. (Significant increases were found in slices from rats treated with equimolar amounts of arginine, citrulline and ornithine in
the presence or absence of ammonium sulfate.)

Reliability: (4) not assignable
insufficient data to allow assessment

10-APR-2006 (106)

In Vitro/in vivo: In vivo
Type: Absorption

Remark: Evidence exists for the active transport of the ammonium ion from the intestinal tract. It was shown that ammonia transport by the human colon still occurred when the luminal pH was reduced to 5, at which value non-ionized ammonia would be virtually absent.

Reliability: (2) valid with restrictions
reliable review

Flag: Critical study for SIDS endpoint

12-APR-2006 (11)

Type: Distribution

Result: Membrane transport of the ammonium ion by the human erythrocyte has been demonstrated.
Reliability: (2) valid with restrictions
older study, older methods
14-JUN-2004 (107)

In Vitro/in vivo: In vivo
Type: Absorption

Result: Administration of an ammonium salt (ammonium chloride, 9 mg/kg bw) 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 at 15 minutes and returned to fasting levels by 30 minutes. However, in 50 male patients with cirrhosis of the liver, blood-ammonia levels increased from already elevated fasting levels to much higher peak concentrations at 15 minutes, 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.
Reliability: (2) valid with restrictions
10-NOV-2003 (108)

In Vitro/in vivo: In vivo
Type: Metabolism

Remark: After intestinal absorption, ammonium ions are primarily transformed by the liver to urea, and subsequently excreted in the urine. Some nitrogen derived from absorbed ammonium is incorporated in amino acids and proteins.
Reliability: (2) valid with restrictions
reliable review
Flag: Critical study for SIDS endpoint
10-APR-2006 (11)

In Vitro/in vivo: In vivo
Type: Distribution
Species: rat

Result: The effect of ammonia intoxication has been studied after intraperitoneal injection of various ammonium salts to rats including ammonium sulfate. A standardized dose of 10.6 mmol NH4-N/kg (1,4 g/kg bw) was lethal within 8-13 minutes for all 13 ammonium salts tested including ammonium sulfate.
Increased levels of NH4-N were detected in blood and brain. A drastic fall in blood pH (7.05) was noted at the moment of

convulsion when ammonium sulfate was injected.
Reliability: (2) valid with restrictions
limited documentation, no further information on purity
18-NOV-2003 (109)

Type: Distribution

Remark: In aqueous environments, such as the body the ammonium sulfate is completely dissociated into the ammonium (NH4+) and the sulfate (SO42-) ions. At physiological pH in aqueous media, the ammonium ion is in equilibrium with un-ionized ammonia, according to the following equation:

$$NH_4^+ + H_2O \text{ --- } NH_3 + H_3O^+$$

The ammonium ion serves a major role in the maintenance of the acid-base balance. In the normal pH range of blood, the NH4+ / NH3 is about 100.
Reliability: (2) valid with restrictions
reliable review
10-APR-2006 (11)

Type: Absorption

Remark: Absorption of sulfate depends on the amount ingested. 30-44% of sulfate was excreted in the 24-h urine after oral administration of magnesium or sodium sulfate (5.4 g sulfate) in volunteers. At high sulfate doses that exceed intestinal absorption, sulfate is excreted in feces. Intestinal sulfate may bind water into the lumen and cause diarrhoea in high doses. Sulfate is a normal constituent of human blood and does not accumulate in tissues. Sulfate levels are regulated by the kidney through a reabsorption mechanism. Sulfate is usually eliminated by renal excretion. It has also an important role in the detoxification of various endogenous and exogenous compounds, as it may combine with these to form soluble sulfate esters that are excreted in the urine.
Reliability: (2) valid with restrictions
reliable review
10-APR-2006 (110)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain: no data
Sex: male/female
Vehicle: other: water (test substance was applied as a 30 % aqueous solution)
Doses: 2500, 3200, 4000, 5000, 6400 mg/kg bw
Value: = 4250 mg/kg bw

Method: other: see Test Condition
Year: 1969
GLP: no

Test substance: other TS: as prescribed by 1.1 - 1.4, "chemically pure"

Result: The LD50 was determined to be 4250 mg/kg bw (95% confidence limits: 3788-4769).
Mortality and time of death(s) per dose group:
6400 mg/kg bw: 12 died within the first hour, 2 within 24 hours, 4 within 48 hours; in total, 18/20 died within 7 days.
5000 mg/kg bw: 8 died within the first hour, 1 within 24 hours, 1 within 48 hours; in total 11/20 died within 7 days.
4000 mg/kg bw: 7 died within 24 hours, and 2 within 48 hours; in total, 9/20 died within 7 days.
3200 mg/kg bw: 1 animal died within the first hour, 1 within 24 hours, 2 within 48 hours; in total, 4/20 died within 7 days.
2500 mg/kg bw: no deaths occurred.
Clinical signs observed were:
4000-6400 mg/kg bw: immediately after application staggering, abdominal and lateral position, partly dorsal position, apathy, laboured and irregular breathing. On the next day, secretion out of eyes and mouth, reddened eyes and nose. In the post-exposure observation days the animals were without clinical symptoms.
3200 mg/kg bw: No symptoms on the application day. The next day, reddened eyes and nose, and irregular breathing were noted. Later without clinical signs.
2500 mg/kg bw: No clinical signs noted.
At necropsy, fluid in the thoracic cavity was observed in a few animals. In three animals, the stomach was filled with liquid, and bloody mouth and forelegs were noted. No pathological findings were noted with regard to the inner organs.

Test condition: According to "Description of methods used in BASF acute toxicity and skin/eye irritation studies before pertinent OECD/EU test guidelines were in place" (BASF AG, 2002)
TEST ANIMALS: Rats of the Gassner strain, mean weight at the beginning of the study 131-148 g.
EXPOSURE TO THE TEST SUBSTANCE:
The test substance was given by single gavage to groups of rats at various dose levels as a 30% aqueous solution. 10 male and 10 female rats were used per dose level.
The LD50 was calculated according to the method described by Litchfield-Wilcoxon.

Reliability: (2) valid with restrictions
post exposure observation period only 7 days, no further information on purity, limited documentation

Flag: Critical study for SIDS endpoint

08-DEC-2003 (111)

Type: LD50
Species: rat
Value: = 2840 mg/kg bw

Reliability: (4) not assignable
secondary citation

17-MAR-2003 (112)

Type: LD50

Species: rat
Strain: Wistar
Sex: male/female
Vehicle: water
Doses: full LD50 test: not reported, -screening test: 2000 mg/kg bw (limit test)
Value: > 2000 mg/kg bw

Method: other: according to TG 423
GLP: no data
Test substance: other TS: ammonium sulfate, commercial grade

Remark: The study was undertaken to validate a screening test for the assessment of the acute oral toxicity with a view to minimizing the use of animals in the acute toxicity testing. Two laboratories cooperated in this study.
Result: Approximative LD50 in males:
in laboratory A > 2000 mg/kg bw,
in laboratory B ca. 2000 mg/kg bw

Approximative LD50 in females:
in laboratory A > 2000 mg/kg bw,
in laboratory B ca. 2000 mg/kg bw.

Clinical signs, necropsy findings: not reported.
Test condition: The full LD50 test (according to the guidelines of Japan) was not performed at this study.
For the screening study, male and female Wistar rats of 5-6 weeks of age were treated with the test substance after at least 5 days of adaptation. The test substance was administered orally in a single dose after fasting for 16 hours.
Observation of animals, including body weight changes, mortality, gross lesion and behavioural and clinical abnormality, were performed for 14 days after exposure, and necropsy was carried out at the end of the test.
The experiments were performed simultaneously in two different laboratories.
Three male and three female rats were each given a dose of 2000 mg/kg bw. If none of the animals died, further toxicity tests were not performed and the chemical was regarded as having an LD50 of greater than 2000 mg/kg bw. If some of the animals died, a step 2 test was performed at the level of 200 mg/kg bw. If all animals survived in the step 2 test, the chemical was considered to have an LD50 greater than 200 mg/kg bw. When some of the animals died, the step 3 test with 20 mg/kg bw was performed. If all the animals died at this dose level, it meant that he chemical had an LD50 of less than 20 mg/kg bw. If one or two animals died at the fixed dose level, the chemical was regarded as having an LD50 near that dose level, and the approximate LD50 of the chemical was indicated as ca. 20, 200 or 2000 mg/kg bw.
Year of study: not reported.
Reliability: (2) valid with restrictions
limited documentation, no further information on purity
Flag: Critical study for SIDS endpoint
11-APR-2006 (113)

Type: LD50

Species: rat
Value: = 3000 - 4000 mg/kg bw

Reliability: (4) not assignable
secondary citation
20-APR-2003 (114)

Type: LD50
Species: mouse
Value: = 640 mg/kg bw

Reliability: (4) not assignable
secondary citation
17-MAR-2003 (112)

Type: LD50
Species: mouse
Strain: other: ddy
Sex: male/female
Vehicle: water
Doses: full LD50 test, 2670-3440mg/kg bw; screening test: 2000 mg/kg bw (limit test)
Value: = 3040 mg/kg bw

Method: other: according to TG 423 (screening) and TG 401 (full test)
Year: 1984
GLP: no data
Test substance: other TS: ammonium sulfate, commercial grade

Remark: The study was undertaken to validate a screening test for the assessment of the acute oral toxicity with a view to minimizing the use of animals in the acute toxicity testing. Two laboratories cooperated in this study.
Result: Results from full LD50 test:
LD50 = 3040 mg/kg bw (2670 - 3440 mg/kg bw)

Results from the screening test:
Approximative LD50 in males:
in laboratory A > 2000 mg/kg bw,
in laboratory B > 2000 mg/kg bw

Approximative LD50 in females:
in laboratory A > 2000 mg/kg bw,
in laboratory B > 2000 mg/kg bw.

Clinical signs, necropsy findings: not reported.
Test condition: The full LD50 test was performed according to the Toxicity Guidelines of Japan (Ministry of Health and Welfare in Japan 1984)
For the screening study, male and female ddy mice of 5-6 weeks of age were treated with the test substance after at least 5 days of adaptation. The test substance was administered orally in a single dose after fasting for 16 hours.
Observation of animals, including body weight changes, mortality, gross lesion and behavioural and clinical abnormality, were performed for 14 days after exposure, and

necropsy was carried out at the end of the test.
The experiments were performed simultaneously in two different laboratories.
Three male and three female mice were each given a dose of 2000 mg/kg bw. If none of the animals died, further toxicity tests were not performed and the chemical was regarded as having an LD50 of greater than 2000 mg/kg bw. If some of the animals died, a step 2 test was performed at the level of 200 mg/kg bw. If all animals survived in the step 2 test, the chemical was considered to have an LD50 greater than 200 mg/kg bw. When some of the animals died, the step 3 test with 20 mg/kg bw was performed. If all the animals died at this dose level, it meant that he chemical had an LD50 of less than 20 mg/kg bw. If one or two animals died at the fixed dose level, the chemical was regarded as having an LD50 near that dose level, and the approximate LD50 of the chemical was indicated as ca. 20, 200 or 2000 mg/kg bw.

Reliability: (2) valid with restrictions
limited documentation, no further information on purity

Flag: Critical study for SIDS endpoint

11-APR-2006 (113)

Type: LD50
Species: mouse
Value: = 2450 - 2500 mg/kg bw

GLP: no
Test substance: other TS: ammonium sulfate, two specimens; purity not stated

Test condition: Two specimens of ammonium sulfate were tested: one was obtained from petrochemical waste products, the other was obtained from ore wastes of the metallurgical industry.

Reliability: (4) not assignable
documentation insufficient for evaluation

17-MAR-2003 (115)

Type: LD50
Species: rabbit
Strain: other: Japanese white
Sex: no data
Vehicle: physiol. saline
Doses: 0; 1500 mg/kg bw
Value: = 1500 mg/kg bw

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Result: All three substance-treated animals showed similar clinical symptoms such as mydriasis, and irregular respiration, beginning at 10-20 minutes after ingestion. Local convulsions persisted for 5-10 minutes in the face or in the extremities after starting at 15 to 25 minutes after ingestion. Soon thereafter they spread to every part of the body, and the animals repeatedly exhibited opisthotonus, each lasting for 10-20 seconds. After the general convulsions, heart rates decreased. 60-70 minutes after ingestion, all rabbits died from cardiac arrest.
The EEG showed typical signs of hyperammonemia, such as

slow, suppressive waves and high-amplitude slowing wave patterns. There was a remarkable increase in the concentration of ammonium ion and inorganic sulfate ion in serum, and blood gas analysis showed severe metabolic acidosis.
Electrolytes did not show any significant change except for a moderate increase in K+ just before cardiac arrest. Concentrations of AST, ALT and LDH remained constant and normal levels were maintained throughout the experiment. Also, the values of BUN and creatinine were always within normal ranges.
No pathological changes were found in brain, heart, lung, spleen, kidney, liver and stomach.

Test condition: Five Japanese white rabbits, weighing 3.4 to 3.8 kg were used. The animals were anesthetized with intravenous pentobarbital sodium (12.5 mg/kg bw), injected in auricular veins, and supplemental doses were intermittently given to maintain surgical level of anesthesia. Rabbits were fixed in supine position and a double channel catheter was placed in the femoral artery to monitor blood pressure and to draw blood samples. Electrocardiogram and electroencephalogram were recorded from needle electrodes. Respiration rate was counted by movement of the thorax. The total dose of 1500 mg/kg ammonium sulfate (dissolved in saline solution) was administered in 10-15 mL of volume per rabbit through gastric probe in three rabbits. 2 rabbits were administered 10-15 mL of saline and used as controls.
Blood samples were collected from the femoral artery every 15 minutes up to 90 minutes after administration of ammonium sulfate solution including 5 minutes before and 5 and 10 minutes after ingestion.
Blood gas was analyzed with heparinized blood and the serum level of ammonium ion, AST, ALT, LDH, BUN and creatinine, as well as electrolytes of Na, K, Cl and Ca were measured. Inorganic sulfate ions in serum were measured by ion chromatography.
Brain, heart, lung, spleen, kidney, liver and stomach were removed after death, and microscopical sections were prepared for histopathological examination with HE- and Elastica Masson stains.
Year of study: not reported.

Reliability: (2) valid with restrictions
limited documentation, only one dose tested

11-APR-2006 (105)

5.1.2 Acute Inhalation Toxicity

Type: other: IRT (inhalatory risk test)
Species: rat
Strain: no data
Sex: no data
No. of Animals: 12
Exposure time: 8 hour(s)

Method: other: see Test Condition
Year: 1969
GLP: no
Test substance: other TS: as prescribed by 1.1 - 1.4, "chemically pure"

Result: No mortality after 8 hours exposure (end of study).
No clinical signs reported.
No pathological findings at necropsy.
Test condition: According to "Description of methods use in BASF acute toxicity and skin/eye irritation studies before pertinent OECD/EU test guidelines were in place" (BASF AG, 2002).
12 rats were exposed for 8 hours to air saturated with the vapor of the test substance at 20 °C. To generate the saturated atmosphere, air was blown through a 5 cm layer of the test substance. Necropsy was performed on all rats at study end.
Reliability: (3) invalid
limited documentation, test system unsuitable for solid substances
08-DEC-2003 (111)

Type: other: effect on the rat respiratory defense system
Species: rat
Strain: Sprague-Dawley
Sex: male
Doses: 3.6 mg/m3 (MMAD: 0.4µm)
Exposure time: 4 hour(s)

Method: other
Year: 1980
GLP: no data
Test substance: other TS: ammonium sulfate

Result: Ammonium sulfate at high or low humidity did not have any significant effects on early or late clearance compared to that in low-humidity clear-air controls.
Test condition: 10-12 rats inhaled 3.6 mg/m3 (0.4 µm) ammonium sulfate as aerosol for 4 hours (in low relative humidity 39 % and high humidity 85%).
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
10-APR-2006 (116)

Species: mouse
Strain: CD-1
Sex: female
Doses: 5 exposure levels, up to 5.3 mg/m3
Exposure time: 3 hour(s)

Method: other: see Test Conditions
GLP: no
Test substance: other TS: (NH4)2SO4, purity not stated

Result: Inhalation of ammonium sulfate had little or no effect on mice in this respiratory infection model in concentrations up to 5.3 mg/m3 (highest tested exposure level). In contrast, inhalation of zinc sulfate (1.2 mg/m3) resulted in significant increases in mortality and reduced survival time.
Mortalities (ammonium sulfate study):
control 233/588 (39.8%, mean survival time 10.1 d),
<1.1 mg SO4/m3: 22/48 (45.8%, mean survival time: 10.1 d),
1.2-2.0 mg SO4/m3: 76/191 (39.8%, mean survival time:10.7

d),
2.1-3.0 mg SO4/m3: 52/96 (54.2%*, mean survival time:9.2 d),
3.1-4.0 mg SO4/m3: 47/144 (32.6%, mean survival time:11.2 d),
5.3 mg (NH4)2SO4/m3: 46/110 (41.8%, mean survival time:10.3 d).
* sign. difference from control, determined by chi-square test, p < 0.05

Test condition: The relative toxicities of zinc sulfate and ammonium sulfate were assessed using a streptococcal model. Mice inhaled varying concentrations of the sulfates for 3 hours and immediately thereafter were challenged with airborne streptococcus.
Between 48 and 144 mice were used per treatment group (ammonium sulfate; 588 mice were used in the control group). For the experiments with zinc sulfate, between 278 and 599 mice were used per group and 1689 mice were used as controls.
Statistical methods: chi-square test for mortalities, Student`s t-test for mean survival time, significance levels at p <= 0.05.

Reliability: (2) valid with restrictions
no common test system

10-APR-2006 (117)

Type: other: mucociliary clearance function
Species: rabbit
Sex: male
No. of Animals: 5
Doses: 2 mg/m3 (MMD 0.4 um)
Exposure time: 1 hour(s)

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Result: The effect of 1 hour oral inhalation exposures to submicrometer aerosols of ammonium sulfate upon mucociliary clearance from the bronchial tree of rabbits was examined. No significant effects were observed at levels up to approximately 2000 ug/m3.
(ammonium bisulfate exposure produced a significant depression of clearance rate at 1700 mg/m3).

Test condition: A group of five male, mixed breed rabbits (O. cuniculus), each weighing 2.5-2.7 kg, were used. All animals were allowed a 1-week quarantine/acclimatization period prior to the experiment.
Throughout the course of the clearance measurements and aerosol exposures, the animals were completely unsedated and were suspended in a sling for these procedures.
MEASUREMENT OF THE MUCOCILIARY CLEARANCE:
The technique involves a brief inhalation of radioactively tagged, insoluble tracer microspheres, with subsequent serial measurement of thoracic retention using external in vivo measurements. the tracer aerosol consisted of monodisperse 99mTc-tagged ferric oxide microspheres (4.5 um MMAD). The serial measurements of retained activity in the lungs were made using an automated system, in which each rabbit is passively restrained within a sling placed between

a pair of opposed, collimated, and electrically coupled scintillation detectors aligned on a common axis on either side of the thorax. Serial measurements were begun within 2 minutes after inhalation of he tracer aerosol. An additional retention measurement was performed 24 hours after tracer exposure.
AEROSOL GENERATION: ammonium sulfate aerosols were generated from dilute (0.03 N) solutions of the chemical using a Laskin nebulizer. The output was mixed with filtered room air which had been temperature and humidity conditioned, and then conveyed into a mixing chamber containing ports for aerosol delivery to the five rabbits. Mass concentration of sulfate was measured in the mixing chamber during each exposure by sampling with Teflon filters, followed by sulfate determination using thermometric titration calorimetry. The size (MMD) of the aerosol was 0.4 um (GSD 1.6).
A series of 10 air sham-control tests (i.e., exposure for 1-hour to temperature and humidity-conditioned air) were performed on each rabbit prior to any sulfate exposure to obtain baseline values. Immediately following each exposure, the rabbit inhaled the tracer aerosol and retention measurements were begun. Tests at each concentration were performed twice.
TEST CONCENTRATIONS: 2000 ug/m3 (nominal), 1800 and 2200 (actual)
STATISTICAL ANALYSIS: paired t test.
YEAR OF STUDY: not reported.

Reliability: (2) valid with restrictions
limited documentation

Flag: Critical study for SIDS endpoint

11-JUN-2004 (118)

Type: other: cardiopulmonary function
Species: dog
Doses: 1.1 to 9.5 mg/m3

Remark: exposure time: 7.5 min to 4 h
Result: Submicron aerosols were administered to groups of 5 anesthetized dogs. Parameters measured included total respiratory resistance by forced oscillations, static lung compliance, specific total respiratory conductance and specific lung compliance. Animals were also exposed to 4.1 mg/m3 for 4 h and additionally functional residual capacity by helium dilution, systemic and pulmonary arterial blood pressures, cardiac output by indicator dilution, heart rate, stroke volume and arterial blood gases were measured at hourly intervals during the exposure and for two hours following discontinuation of aerosol administration.
Analysis of variance revealed no statistically significant differences between sodium chloride and ammonium sulfate aerosol in any of the parameters measured.

Reliability: (2) valid with restrictions
unusual animal model, but data well documented

Flag: Critical study for SIDS endpoint

11-APR-2006 (119)

Type: LC50

Species: guinea pig
Strain: no data
Sex: no data
Doses: 500-900 mg/m3, average particle size 1-3 um
Exposure time: 8 hour(s)
Value: > 900 mg/m³

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Remark: maximum attainable concentration of ammonium sulfate using the equipment and methods available were used.
Result: 8/20 guinea pigs exposed to 800-900 mg/m3, 1/6 exposed to 600-700 mg/m3 and 0/6 exposed to 500-600 mg/m3 died during exposure. The authors gave acute shock and airway constriction as explanation for this effect. After exposure, the survivors recovered with no noticeable after effects.
Test condition: Twenty guinea pigs were exposed (whole body) for a single 8-hours period to a concentration of 800-900 mg/m3, six to a concentration of 600-700 mg/m3, and six to a concentration of 500-600 mg/m3. The animals were observed for mortality and signs of gross toxicity.
Ammonium sulfate aerosol was generated from an aqueous solution with Retec nebulizers and dried by mixing with dry air and passing it through a heated glass tube. Ammonium sulfate concentration was determined by collecting the aerosol on a glass fiber filter at a flow rate of 2 L/min for 15 minutes and weighing the filter. Accuracy of the method was periodically checked by chemical analysis of the filter sample. Particle size was evaluated gravimetrically using an Andersen multi-stage sampler.
Reliability: (2) valid with restrictions
limited documentation, no information on purity, particle size distribution not given
Flag: Critical study for SIDS endpoint
10-APR-2006 (120)

Type: other: effect on pulmonary function
Species: guinea pig
Strain: no data
Sex: no data
Doses: 0.5 - 9.5 mg/m3, 4 groups
Exposure time: 1 hour(s)

GLP: no
Test substance: other TS: (NH4)2SO4, purity not stated

Result: A slight increase in pulmonary flow resistance and a statistically significant decrease in pulmonary compliance at all concentrations and particle sizes tested was found. On a scale in which the percentage increase in resistance caused by 0.3 um H2SO4 particles was assigned the value of "100", ammonium sulfate was assigned a value of "10", i.e. a value in-between NH4HSO4 (value of 3), and ZnSO4 (value of 19).
Test condition: mass median diameter (MMD): 0.1 - 0.8 um.
10 animals per group
Reliability: (2) valid with restrictions
no information on purity, no common test system

Flag: Critical study for SIDS endpoint
21-JUN-2004 (121)

Type: other: pulmonary functional response
Species: guinea pig
Vehicle: other: Aerosol
Doses: 1 mg/m3 (0.7um MMAD)
Exposure time: 1 hour(s)

Method: other
Year: 1977
GLP: no
Test substance: other TS: Ammonium sulfate

Method: 12 female guinea pigs inhaled via intratracheal catheter 1 mg/m3 ammonium sulfate (MMD 0,7 um) for 1 hour. The recovery time was 1/2 hour. 14 control animals were exposed with to air only. Lung function measurements were performed. Dynamic lung compliance (Cdyn) and pulmonary flow resistance (RL) were calculated.
The animals were killed and examined (lungs for evidence of gross injury caused by catheter and for diseases).
The average values of the functional measurements for the control, exposure, and recovery time were reported.
Result: The comparison of Cdyn and RL values during control period and exposure within each animal showed changes of these parameters in a fraction of the exposed population.
The changes were only slight and comprised increases and decreases.
Overall a decrease in Cdyn of 2% and an increase in RL of 7% were found.
Reliability: (2) valid with restrictions

no common test system, high interanimal variability, no information on test substance purity.
10-APR-2006 (122)

Type: other: effect on pulmonary function
Species: other: donkey
Strain: no data
Sex: no data
Doses: 0.4 - 2.1 mg/m3
Exposure time: 1 hour(s)

Method: other: see Test Conditions
GLP: no
Test substance: other TS: (NH4)2SO4, purity not stated

Remark: Because of the great variability in the intraanimal control measurements, a change in flow resistance of more than 25% was needed to detect a significant alteration caused by the exposure.
Result: No effects on pulmonary flow resistance or dynamic compliance were seen.
Test condition: 4 donkeys were exposed to ammonium sulfate aerosols (0.4-2.1 mg/m3) for 1 hour.
The median aerodynamic diameter (MAD)was 0.4 um.
Reliability: (3) invalid

not common test system
22-JUL-2004 (123)

Type: other: effect on pulmonary function
Species: sheep
Sex: no data
Doses: 0.1 and 4.0 mg/m3, both concentrations tested at MMAD of 0.5 and 1.5 um
Exposure time: 4 hour(s)

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Result: Pulmonary flow resistance was not affected by any aerosol tested.
At 4.0 mg/m3, the airway responsiveness was enhanced only when comparing challenge immediately or 24 h after ammonium sulfate exposure to baseline values and when tested at a MMAD of 1.5 um, but not at 0.5 um.

Test condition: The effects of ammonium sulfate aerosols on pulmonary resistance flow and airway responsiveness to inhaled carbachol were studied in conscious sheep.
TEST ANIMALS: 28 adult ewes with a mean weight of 34 kg (range 25-46 kg), 6-8 animals per test group.
MEASUREMENT OF RESPIRATORY MECHANISMS:
The unsedated, restrained sheep were intubated and a catheter was placed into the lower esophagus for the estimation of pleural pressure. Lateral pressure in the trachea was measured with a sidehole catheter advanced through and positioned distal to the tip of the endotracheal tube. Mean pulmonary flow resistance was measured by connecting the nasotracheal tube to a pneumotachograph.
AIRWAY RESPONSIVENESS:
Baseline pulmonary flow resistance was obtained after inhalation of phosphate buffer. Bronchial challenges were then performed using aerosolized solutions of phosphate buffered carbachol. The airway responsiveness was defined as the percentage increase from the baseline. Half an hour after determination of airway responsiveness, animals were exposed 4 h to ammonium sulfate aerosol and immediately after exposure and 24 h later baseline pulmonary resistance and airway responsiveness were remeasured.
AEROSOL GENERATION:
A commercially available ultrasonic nebulizer was modified for long term stability and used for the generation of two particle sizes, MMAD 0.5 um and 1.5 um. Each particle size was delivered at concentrations of 4 and 0.1 mg/m3. Humidity was kept at 40%.
EXPOSURE TO TEST SUBSTANCE:
The sheep breathed the sulfate aerosols for 4 hours (head only exposure). Some sheep were exposed to more than one salt and in these instances the exposure were separated by at least one week.

Reliability: (3) invalid
limited documentation, high interanimal variability, the animals showed no reaction to H2SO4
10-APR-2006 (124)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rat
Strain: Wistar
Sex: male/female
Vehicle: other: water-acetone solution (no further detail reported)
Doses: 2000 mg/kg bw (limit test)
Value: > 2000 mg/kg bw

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulphate, commercial grade

Remark: The study was undertaken to validate a screening test for the assessment of the acute dermal toxicity with a view to minimizing the use of animals in the acute toxicity testing. Two laboratories cooperated in this study.

Result: Approximative LD50 in males:
in laboratory A > 2000 mg/kg bw,
in laboratory B > 2000 mg/kg bw.

Approximative LD50 in females:
in laboratory A > 2000 mg/kg bw,
in laboratory B > 2000 mg/kg bw.

Clinical signs, necropsy findings: not reported.

Test condition: Male and female Wistar rats of 5-6 weeks of age were treated with the test substance after at least 5 days of adaptation. Hair was first removed from an area of 3x4 cm2 on the back with an electric hair clipper, and then the test substances dissolved in acetone and water were applied in a single dose to the skin surface of the clipped backs of the animals. The application sites were not covered but the treated areas were prevented from being licked by using a plastic collar or by fixing the animals on a plastic plate, and all animals were individually housed in stainless-steel cages. Observation of animals, including body weight changes, mortality, gross lesion and behavioural and clinical abnormality, were performed for 14 days after exposure, and necropsy was carried out at the end of the test.
The experiments were performed simultaneously in two different laboratories.
Three male and three female rats were each given a dose of 2000 mg/kg bw. If none of the animals died, further toxicity tests were not performed and the chemical was regarded as having an LD50 of greater than 2000 mg/kg bw. If some of the animals died, a step 2 test was performed at the level of 200 mg/kg bw. If all animals survived in the step 2 test, the chemical was considered to have an LD50 greater than 200 mg/kg bw. When some of the animals died, the step 3 test with 20 mg/kg bw was performed. If all the animals died at this dose level, it meant that he chemical had an LD50 of less than 20 mg/kg bw. If one or two animals died at the fixed dose level, the chemical was regarded as having an LD50 near that dose level, and the approximate LD50 of the chemical was indicated as ca. 20, 200 or 2000 mg/kg bw.
Year of study: not reported.

Reliability: (2) valid with restrictions
limited documentation, open application, no further information on purity
Flag: Critical study for SIDS endpoint
24-AUG-2005 (113)

Type: LD50
Species: mouse
Strain: other: ddy
Sex: male/female
Vehicle: other: water-acetone solution (no further detail reported)
Doses: 2000 mg/kg bw (limit test)
Value: > 2000 mg/kg bw

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulphate, commercial grade

Remark: The study was undertaken to validate a screening test for the assessment of the acute dermal toxicity with a view to minimizing the use of animals in the acute toxicity testing.

Two laboratories cooperated in this study.
Result: Approximative LD50 in males:
in laboratory A > 2000 mg/kg bw,
in laboratory B > 2000 mg/kg bw.

Approximative LD50 in females:
in laboratory A > 2000 mg/kg bw,
in laboratory B > 2000 mg/kg bw.

Clinical signs, necropsy findings: not reported.
Test condition: Male and female ddy mice of 5-6 weeks of age were treated with the test substance after at least 5 days of adaptation. Hair was first removed from an area of 1x2 cm2 on the back with an electric hair clipper, and then the test substances dissolved in acetone and water were applied in a single dose to the skin surface of the clipped backs of the animals. The application sites were not covered but the treated areas were prevented from being licked by using a plastic collar or by fixing the animals on a plastic plate, and all animals were individually housed in stainless-steel cages.
Observation of animals, including body weight changes, mortality, gross lesion and behavioural and clinical abnormality, were performed for 14 days after exposure, and necropsy was carried out at the end of the test.
The experiments were performed simultaneously in two different laboratories.
Three male and three female mice were each given a dose of 2000 mg/kg bw. If none of the animals died, further toxicity tests were not performed and the chemical was regarded as having an LD50 of greater than 2000 mg/kg bw. If some of the animals died, a step 2 test was performed at the level of 200 mg/kg bw. IF all animals survived in the step 2 test, the chemical was considered to have an LD50 greater than 200 mg/kg bw. When some of the animals died, the step 3 test with 20 mg/kg bw was performed. If all the animals died at this dose level, it meant that he chemical had an LD50 of less than 20 mg/kg bw. If one or two animals died at the

fixed dose level, the chemical was regarded as having an LD50 near that dose level, and the approximate LD50 of the chemical was indicated as ca. 20, 200 or 2000 mg/kg bw.
Year of study: not reported.

Reliability: (2) valid with restrictions
limited documentation, open application, no further information on purity

Flag: Critical study for SIDS endpoint

24-AUG-2005 (113)

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: mouse
Strain: no data
Sex: male/female
Vehicle: other: the test substance was applied as 8% aqueous solution
Doses: no data
Route of admin.: i.p.
Value: 900 mg/kg bw

Method: other: see Test Condition
Year: 1969
GLP: no
Test substance: other TS: as prescribed by 1.1 - 1.4, "chemically pure"

Result: The LD50 was determined to be 900 mg/kg bw (95% confidence limits: 608-1332).
Clinical signs observed were: dyspnoea, tremor, palpitations, cramps, and prostration at dose levels near to or exceeding the LD50.
At necropsy, no pathological findings were noted.

Test condition: According to "Description of methods used in BASF acute toxicity and skin/eye irritation studies before pertinent OECD/EU test guidelines were in place" (BASF AG, 2002).
The test substance was applied as 8% aqueous solution to groups of mice as single intraperitoneal injection at various dose levels. The LD50 was calculated according to the method described by Litchfield-Wilcoxon.
Post exposure period: 7d

Reliability: (2) valid with restrictions
limited documentation, post exposure observation period only 7 days

18-NOV-2003 (111)

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: = 610 mg/kg bw

Method: other: no data
GLP: no data
Test substance: other TS: no data

Reliability: (4) not assignable
secondary citation

09-MAR-2003 (125)

Type: LD100
Species: rat
Strain: Wistar
Sex: male
Route of admin.: i.p.

Test substance: other TS: ammonium sulfate, reagent grade

Result: The effect of ammonia intoxication has been studied after intraperitoneal injection of various ammonium salts to rats including ammonium sulfate. A standardized dose of 10.6 mmol NH4-N/kg (1,4 g/kg bw) was lethal within 8-13 minutes for all 13 ammonium salts tested including ammonium sulfate.
Reliability: (2) valid with restrictions
limited documentation. No further information on purity.
18-NOV-2003 (109)

Type: other: pulmonary oedema
Species: rat
Strain: Wistar
Sex: male/female
No. of Animals: 19
Doses: 6 % (600 mg/kg bw)
Route of admin.: i.p.

Method: other
Year: 1969
GLP: no data
Test substance: other TS: ammonium sulfate

Result: The major lesion was the formation of large blebs in endothelial cells, associated with a variable degree of oedema of the interstitium and of epithelial lining cells. These changes were present in every animal exposed to ammonium sulfate, irrespective of the time-interval after injection, the only difference being that the changes prominent in those exposed for longer intervals.
The ultrastructural changes, produced by ammonium sulphate are similar to the changes in pulmonary oedema induced by methods other than by altered haemodynamics. The absence of carbon-marker leakage through intercellular junctions in this experiment suggest that the increased vascular permeability necessary for the formation of pulmonary oedema differs from leakage occurring in acute inflammation, in quality if not in mechanism.
Test condition: 19 rats were used. A colloidal carbon suspension was injected into the tailveins of the rats in a dosage of 0.1 ml per 100 g bw. immediately after, a 6 % solution of ammonium sulfate was injected i.p. in a dosage of 10 ml/kg bw. Rats were killed at varying intervals (1.5, 5, 7.5, 10, 15, 20, 30, 90 min) after injection of ammonium sulfate. 9 control animals were used. The lungs were prepared and examined by electron microscopes. Other organs like liver, spleen, kidney, heart and skeletal muscle were prepared and stained as a routine with haematoxylin and eosin, and in some instances with Weigert´s elastic tissue stain or by the periodic acid-Schiff method and examined microscopically or stained with uranyl acetate and examined by electron microscopy.

Reliability: (2) valid with restrictions
no common test system
10-APR-2006 (126)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration: 80 %
Exposure: Occlusive
Vehicle: water
Result: not irritating

Method: other: see Test Condition
Year: 1969
GLP: no
Test substance: other TS: as prescribed by 1.1 - 1.4, "chemically pure"

Result: Barely visible skin erythema (equivalent to a Draize score of 0) was noted at 24 hours after exposure when test substance was applied for 5 minutes, 15 minutes or 20 hours. No effects were noted at 8 days after the end of the exposure or after exposure to the test substance for only 1 minute. Neither scaling nor edema were noted at any observation time.
No mortality occurred and there were no clinical signs of toxicity observed.
Test condition: According to "Description of methods used in BASF acute toxicity and skin/eye irritation studies before pertinent OECD/EU test guidelines were in place" (BASF AG, 2002)
TEST ANIMALS: Rabbits, White Vienna strain, 2 animals. Each animal was treated with the test substance as 80% aqueous preparation for 1 minute, 5 minutes, 15 minutes and 20 hours.
The test substance was applied under occlusive dressing to the back skin of the animals.
OBSERVATIONS: the treated skin was evaluated for irritant effects at the end of the exposure, i.e. at 1 minute, 5 minutes, 15 minutes and 20 hours and at 24 hours and 8 days after start of the study.
Reliability: (2) valid with restrictions
limited documentation, application time not according to OECD TG 404
Flag: Critical study for SIDS endpoint
08-DEC-2003 (111)

Species: rabbit
Concentration: undiluted
Exposure: Semiocclusive
Result: not irritating

Method: other: see Test Condition
Year: 1968
GLP: no
Test substance: other TS: ammonium sulfate, about 0.1% water content,

different batches (cf Test Condition).

Result: Single exposure for 20 hours, intact skin: no skin effects observed.

Multiple exposures, 8 hrs/day for 5 consecutive days, intact skin: no skin effects observed.

Single exposure for 8 hours, scarified skin: slight redness, slight edema; completely reversible within 8 days. NaCl induced strong erythema, strong edema and necroses, and distilled water slight redness in these experiments.

Test condition: The neat test substance (about 1.0 g) was applied on moistened patches (about 2.5 x 2.5 cm in size) to the intact back skin of white rabbits. Exposure time was either 20 hours or 8 hours each on 5 consecutive days. In another test the test substance was applied in the same manner as described above to scarified skin for 8 hours. Skin treated with distilled water or NaCl served as control in the experiments on scarified skin.
The tests on intact skin were performed with 12 different batches of the test substance. Four of the batches contained 0.03% anti-caking material.
The tests on scarified skin were performed with 8 different batches of the test substance. None of the batches contained anti-caking material.
Number of animals tested: not reported.

Reliability: (2) valid with restrictions
limited documentation, no information on purity

Flag: Critical study for SIDS endpoint

18-NOV-2003 (127)

5.2.2 Eye Irritation

Species: rabbit
Concentration: undiluted
Dose: 50 other: mm3
Vehicle: none
Result: not irritating

Method: other: see Test Condition
Year: 1969
GLP: no
Test substance: other TS: as prescribed by 1.1 - 1.4, "chemically pure"

Result: Slight edema and conjunctival redness was noted at 1 hour after instillation of the test substance; no edema, but still slight redness was present at 24 hours. In the eyes treated with talcum, slight redness was also noted at 1 and 24 hours after exposure. No effects were noted at day 8.

Test condition: According to "Description of methods used in BASF acute toxicity and skin/eye irritation studies before pertinent OECD/EU test guidelines were in place" (BASF AG, 2002)
TEST ORGANISMS: Rabbits, White Vienna strain. 2 Animals.
EXPOSURE TO TEST SUBSTANCE:
Undiluted test substance was instilled into one eye of rabbits, the other eye was treated in the same way with 50 mm3 of talcum and served as control. The eyes were not

rinsed.
OBSERVATIONS: Eyes were examined at 1 hour, 24 hours and several times thereafter until 8 days after instillation.

Reliability: (2) valid with restrictions
limited documentation, no further information on purity

Flag: Critical study for SIDS endpoint

10-APR-2006 (111)

5.3 Sensitization

5.4 Repeated Dose Toxicity

Type: Sub-acute
Species: rat **Sex:** male
Strain: no data
Route of administration: inhalation
Exposure period: up to 14 days
Frequency of treatment: 8 hours/day
Post exposure period: no
Doses: 300 mg/m3
Control Group: other: air

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Result: No effects on arterial blood gases, pH and bicarbonate levels in blood were found. No effect was noted on residual volume and vital capacity of the lung. Trachea, bronchial lymph nodes and lungs showed no histopathological changes. The body weights of the animals were unaffected.As the respiratory tract is the target organ, the NOEL for the lower respiratory tract is 300 mg/m3

Test condition: The study was designed to measure arterial blood gases in rats (10 adult males/group) after inhalation of 300 mg/m3 ammonium sulfate for 1, 3, 7 or 14 days, 8 h/day. Pulmonary function tests were performed in animals after 14 days of exposure.
Microscopic examinations of the lung were also performed.
The concentrations used were determined on basis of the results from a preliminary study with rats were exposed to the maximum attainable concentration of ammonium sulfate using the same equipment and methods, i.e. a group of 6 male rats were exposed 8 h/day for 3 consecutive days to a concentration of 1000-1200 mg/m3. No toxicological effects were noted in this pretest.
Ammonium sulfate aerosol was generated from an aqueous solution with Retec nebulizers and dried by mixing with dry air and passing it through a heated glass tube. Ammonium sulfate concentration was determined by collecting the aerosol on a glass fiber filter at a flow rate of 2 L/min for 15 minutes and weighing the filter. Accuracy of the method was periodically checked by chemical analysis of the filter sample. Particle size was evaluated gravimetrically using an Andersen multi-stage sampler. The particle size was between 1 and 2 um in diameter.

Conclusion: Exposure to levels of 300 mg/m3 (mean diameter 1-2 um) for up to 8 hours per day in 14 days did not result in any significant changes in lung morphology, lung volumes and arterial blood gases.
Reliability: (4) not assignable
limited documentation, no information on purity and particle size distribution (SD), only lung, trachea and bronchial lymphnodes examined, only one dose tested.
The interval between termination of exposure and examination was too long.
Flag: Critical study for SIDS endpoint
25-AUG-2005 (120)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 4 weeks
Frequency of treatment: 6 hours/day, 5 days/week
Post exposure period: no
Doses: 1.03 mg/m3 (MMAD 0.42 um)
Control Group: other: sham exposed

Remark: The study was designed to investigate effects of ammonium sulfate in normal animals and animals pretreated with elastase, which results in lung emphysema. Only the results for ammonium sulfate vs. air controls are reported in detail.
Result: Pathological examination of rats exposed for 20 days to 1.03 mg/m3 ammonium sulfate aerosol revealed a measurable degree of enlargement of alveoli, alveolar ducts and sacs. Alveolar pore size was not increased.
The authors concluded that elastase pretreatment did not enhance or even obscure the effect of ammonium sulfate on alveolar structure at these exposure levels after short-term treatment.
Test condition: Young adult specific pathogen free animals were maintained in isolation for 3 weeks prior to beginning experimental procedures. Animals were assigned to four experimental groups based on their initial weights, so that the distribution of animal weights for each treatment group was the same. The animals were divided into two groups, one to receive intratracheally instilled porcine pancreatic elastase ("elastase impaired") and one to receive saline solution intratracheally (controls). 40 rats received 75 units of elastase activity / 100 g bw. The dose level was determined in preliminary experiments to produce a predictable degree of emphysema without inducing significant mortality. 40 rats received saline.
Sterile elastase or saline solutions, standardized for activity (in the case of elastase), osmolarity, pH, and temperature, were administered intratracheally to ether-anesthetized animals. The total volume of the instillate varied slightly, depending on animal weight; however, concentration/volume was constant. Following recovery from anesthesia, the animals were caged for a 3-week recovery period, during which (as in the initial isolation period) rats were maintained on tetracycline administered via drinking water. This procedure was found

necessary, during preliminary experiments, to prevent changes resulting from pneumonia (and subsequent mortality).

EXPOSURE TO TEST SUBSTANCE:
One-half of the elastase-treated animals and one-half of the saline-treated animals were exposed to ammonium sulfate, and the remainder were exposed to air only. Animals were exposed and housed in Hazelton Systems Model 100 stainless-steel exposure chambers. The chamber air flow of 0.30 m3/min provided seven changes of filtered air per hour.
Aerosols were introduced into and thoroughly mixed with the filtered air stream before entering the chambers. Several Retec nebulizer generators supplied with special reservoirs provided a constant ammonium sulfate solution concentration and liquid level for stable aerosol generation.
Time-weighted average concentrations of aerosol were determined from four 90-mm filter samples taken over the daily 6-hr exposure periods. These filter samples were analysed, chemically and gravimetrically, and corrected by correlating the amount of particles recovered by spiked filters with each set of samples. Daily time-weighted-average concentrations for ammonium sulfate were 1.03 mg/m3 +/- 0.11 SD (gravimetric) and 0.97 mg/m3 +/- 0.11 SD (chemical). Aerosol particle size was measured by a cascade impactor (Mercer design) on samples collected over 15 hours of exposure time during a 5-day week. The MMAD was 0.42 +/- 0.05 um SD, geometric standard deviation (GSD) was 2.25 +/- 0.22.
Animals were exposed for 6 hours/day, 5 days/week, for a total of 20 exposures. Control (air only) animals were maintained in identical chambers under similar conditions of air flow, temperature, humidity, cage changes, etc. All animals were housed in similar chambers during nonexposure periods.
At the end of the exposure period (4 weeks after intratracheal instillations), animals were deeply anesthetized, and the lungs perfused with 2% glutaraldehyde solution. The lungs were removed for examination after observation of macroscopic features in situ. Fixed lung volume was determined by weight of displaced fluid.
Slices of approximately 1 mm were taken from both the right diaphragmatic and left lobes of each animal and examined under a dissecting microscope for the degree of emphysema. A subjective rating of 0 (none observed) to 5 (total involvement) was given to each tissue slice. The rating (score) was determined by estimating the proportion of the slice affected by disruptive alveolar changes and the severity of alveolar disruption within involved areas. The area affected was determined.
From each animal, the slice most closely approximating the mean degree of involvement was prepared for examination by scanning electron microscopy (SEM).
Morphometry: Chord lengths were measured across alveoli, alveolar sacs, and alveolar ducts from photographs taken during SEM. The median, mean, and SD were calculated for these chord length measurements, and histograms were developed. To ensure consistency in measurements between photographs the criteria as developed by Busch et al.,1984 (Env. Res. 33, 497-513) were followed. In addition, alveolar

pores, the number of nonciliated bronchiolar epithelial cells per standardized area was counted, and means and SD were calculated.
A section of the right cardiac lobe was prepared for examination by light microscopy to identify any pathologic processes.
Statistical analysis: two-way analysis of variance.

Reliability: (4) not assignable
no further information on purity, only one dose tested.
11-APR-2006 (128) (129)

Type: Sub-chronic
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 4, 8 months
Frequency of treatment: 5 hours/d, 5 d/w
Post exposure period: half of the rats exposed for 8 months were maintained for an additional 3-month recovery period
Doses: 0.5 mg/m3 (MMAD 0.44 um)
Control Group: yes, concurrent no treatment

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, no further details given

Remark: The study was designed to investigate effects of ammonium sulfate in normal animals and animals pretreated with elastase, which results in lung emphysema. Only the results for ammonium sulfate vs. air controls are reported in detail.

Result: No significant differences were found in final body weights or some immunologic parameters measured after 4 months only (spleen weight, mitogenic response of spleen cells or peripheral blood lymphocytes, distribution of T cells in the spleen). No differences were found in vital capacity, total lung capacity, time constant, CO2 diffusion capacity, residual volume, functional residual capacity or N2-slope. The quasistatic compliance was reduced significantly. Only after 4 months was the incidence and severity of bronchiolar epithelial hyperplasia significantly increased. Non ciliated epithelial cell counts were increased significantly after 4 and 8 months. Chord length means and medians were increased after 4 months significantly but only numerically after 8 months. However, after the recovery period, these differences were significant. After 8 months, emphysema incidence and severity were increased only numerically whereas alveolar interstitial birefringence, a measure of fibrosis, was increased significantly. Elastase pretreatment seems to have repressed or obscured the effects of ammonium sulfate.

Morphological lung effects in saline-pretreated rats after exposure to ammonium sulfate (AS) for 4 months, 8 months, or 8 months plus 3 months recovery:

Exposure	4 months		8 months		8 + 3 months	
N	Air	AS	Air	AS	Air	AS

Emphysema	/ 2/15	0/15	/ 0/13	2/15 /	2/13	4/14 /
Alveolar. B	/ 8/15	0/15	/ 9/13	15/15*/	10/13	12/14 /
Bronch. EH	/ 6/15	13/15*	/ 0/13	0/15 /	0/13	0/14 /
NEC** counts	/ 27.7	32.7*	/ 24.8	30.0*/	26.4	25.9 /
Chord length: Mean (mm)	/ 4.66	5.07*	/ 4.28	4.45 /	3.87	4.14*/
Chord length: Median(mm)	/ 4.29	4.47*	/ 3.71	3.99 /	3.60	3.92*/

N, Number of animals with effects/total number of animals
Alveolar. B, alveloar birefringence
Bronch. EH, bronchiolar epithelial hyperplasia
*, $p < 0.05$
**, non-ciliated epithelial cells/standard area

Test condition: AEROSOL ANALYSES: The overall mean values and standard deviations of the 190 daily average concentrations were 0.496 +/- 0.027 mg/m3 for the ammonium sulfate-only exposures, with minimum and maximum daily mean concentrations of 0.38 and 0.67 mg/m3.
The 40-week mean particle size distribution was 0.44 um (MMAD), GSD 2.2. The percentage of aerosol mass in particles equal to or smaller than 0.92 um averaged 86.1 +/- 3.15% for the ammonium sulfate-only exposures.
Young adult specific pathogen free animals from Charles River Labs., Portage, MI) were used. To control exposure conditions as closely as possible, animals were introduced into chambers at intervals. Two shipments were spaced so as to provide animals comparable in age and weight for each exposure grouping. The first shipment of rats was used for morphologic evaluation and pulmonary function tests, and the second shipment was used for immunology studies. The rats were initially maintained in isolation and weighed weekly for 3 weeks, after which they were randomly assigned to experimental groups so that no statistically significant differences existed among initial group mean body weights. Each rat was first assigned to one of two main groups for pretreatment (for normal and elastase-impaired lungs), after which its exposure group was designated.
ANIMAL PRETREATMENT: 60 ether-anesthetized rats were pretreated with porcine-pancreatic elastase (EO 127, Sigma Chemical Co., St. Louis, MO) by intratracheal instillation of 28 units elastase activity/ 100 g bw. one unit of elastase activity was defined as the amount of enzyme which hydrolyzed 1.0 mg of elastin-orcein at 37 degree C, at pH 8.8, in 20 min. The total volume of the instillate was approximately 1 mL but varied slightly with animal weight. An additional 60 rats were similarly pretreated with physiologic saline. Animals designated for immunological tests did not receive the saline/elastase pretreatment. Following pretreatment, the animals were held for a 3-week recovery period, during which (as in the initial isolation period) they were maintained on tetracycline-treated

drinking water. This procedure was found necessary to prevent lung changes and mortality from pneumonia.
EXPOSURE TO THE TEST SUBSTANCE: Half of the pretreated animals of both groups were exposed to ammonium sulfate (0.5 mg/m3), the other half to filtered air (controls).
The rats were housed and exposed in Hazleton Systems model 1000 stainless steel chambers in compliance with the National Institutes of Health guidelines (NIH, 1978). They were maintained on a 12-hr light/dark cycle. Food (Wayne Labs Animal Diet) and water were available ad lib. Relative humidity was maintained at 50-60%. Exposures were for 5 hours/day, 5 days/week, for either 4 or 8 months. Half of the rats exposed for 8 months were sacrificed immediately; the remaining half were held for an additional 3-month recovery period. The rats were weighed at 2-week intervals throughout exposure and recovery period.
MORPHOLOGY AND MORPHOMETRY: For sacrifice, animals were deeply anesthetized by ip injection of pentobarbital sodium and weighed. The lungs were perfused in situ with cacodylate-buffered 2% glutaraldeyhde at 20 cm H20 pressure. The lungs were removed and stored in the glutaraldehyde solution. The nasal passages wee gently flushed with 10% neutral buffered formalin (NBF), and the heads (with skin, brain, and mandibles removed) were fixed in NBF. The fixed lung volume was determined by weight of displaced fluid. Lung slices were examined by scanning electron microscopy (SEM) and alveolar chord length measurements as well as determinations of the number of nonciliatated bronchiolar epithelial cells were performed according to previously described methods (Busch et al., 1984). Three sections of the nose and one section from the right cardiac lung lobe were examined by light microscopy.
PULMONARY FUNCTION: Anesthetized animals were intubated with an esophageal catheter and an endotracheal tube and placed in a flow-type plethysmograph. Measurements on spontaneously breathing rats utilized a syringe to inflate and evacuate the lungs. Transpulmonary pressure and total lung capacity were recorded. Residual volume was determined by inert gas dilution (0.5% neon in air). Single-breath carbon monoxide diffusion capacity was determined according to the procedure of Takezawa et al (1980, J.App.Physiol. 48, 1052-1059) with modifications (Loscutoff,1985, Environmental Research 36, 170-180.), and the time for a single breath was calculated. Time constant (the time required for lung volume to decrease by 63% of the total exhaled volume) was measured by inflating the lungs to total lung capacity and allowing them to exhale passively. Quasistatic compliance and single-breath N2 washout were measured in succinyl choline paralyzed rats by inflating the lungs to total lung capacity with air or O2 respectively, and deflating to residual volume at a flow rate of 2.5 mL/sec. Functional residual capacity was measured in paralyzed rats by inert gas dilution.
IMMUNOLOGIC STUDIES: These studies included cellular measurements that correlate with immune competence. Mitogen-induced activation of peripheral blood lymphocytes and spleen cells, assayed by incorporation of 125I-iododeoxyuridine into newly synthesized DNA was used as an in vitro measure of cell-mediated immunity. The distribution of the T-cell population in spleen cell

preparations was determined by the selective incorporation of tritium-uridine. Four T- and B-cell specific mitogens were utilized for the mitogen-induced activation test (concanavalin A (Con A), phytohemagglutinin (PHA), pokeweed mitogen (PWM), lipopolysaccharide (LPS)).
STATISTICAL ANALYSES. two-way analysis of variance, analysis of covariance, Fisher´s exact test. Immunologic studies did not involve comparisons of lung condition, and only the differences among the four exposure groups were tested by analysis of variance.

Conclusion: A series of studies in which rats and guinea pigs were exposed to 1 or 0.5 mg/m3 for up to 8 month describes histomorphometrically determined increases in emphysema formation, hyperplasia of non-ciliated cells in the bronchiolar epithelium and increased interstitial birefringence indicating increased collagen content. The findings showed a high variation within the control animals and no consistent time-response pattern. There was no close correlation between the morphological findings and lung function tests, most of which did not show differences between control and treated groups. Therefore these findings cannot definitively be attributed to the test substance treatment. This conclusion is supported by the fact that ammonium sulfate is a highly soluble material with a moderately acidic pH (around 5), which is rapidly neutralized and absorbed when deposited in the respiratory tract. It is thus not expected to show cytotoxicity.

Reliability: (4) not assignable
no information on purity, only one dose tested.
11-APR-2006 (128) (130)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 23.5 h up to 7 days
Doses: 5 mg/m3 as aerosol

Result: The apparent lung collagen synthesis rate, assayed by the incorporation of tritium labeled proline into hydroxyproline of lung collagen, was increased to 35 nmol/g at 0.64 ppm ozone, which was about 200% above the control values. Total protein content of bronchoalveolar lavage reached a maximum of about 10 mg, a 590% increase over the control levels after a 1 day (23.5h) exposure to 0.64 ppm of ozone. The level declined to near base amounts after 7 days. Lower ozone doses elicited proportionally smaller increases in the total protein. The lung protein content increased to about 140 mg at the high ozone level after 7 days. The lung DNA content was elevated to 125% over the base amount at 0.64 ppm, the 0.12 and 0.20 ppm doses showed no increase. The exposure to 0.20 ppm of ozone and about 5 mg/m3 of ammonium sulfate resulted in a 125% increase in collagen synthesis. Exposures to 5 mg/m3 of Ammonium sulfate alone have been shown previously not to cause changes in collagen synthesis rate as compared with control. Seven day exposure to the high level of ozone increased the

activities of lactate-dehydrogenase, acid-phosphatase, and N-acetyl-beta-D-glucosaminidase 135, 135, and 155%, respectively. The addition of ammonium sulfate caused no significant change from the above values with ozone, ammonium sulfate had no synergistic effect on tissue protein or DNA content.
Exposure to ammonium sulfate alone did not increase the protein content of the BAL.

The described synergistic effect of ozone and ammonium sulfate seems to be questionable, since only the middle dose of 0.2 ppm ozone was tested for this endpoint, it is not stated if the other ozone concentrations were not tested or did not show an effect. From the discussion one might interpret that also the combination of 0.64 ppm ozone and ammonium sulfate was tested without showing a synergistic effect.

Test condition: Pulmonary changes in rats induced by ozone and the synergistic effects of ammonium sulfate were studied.
Male Sprague-Dawley rats were exposed to 0.12, 0.20, 0.40, and 0.64 ppm of ozone and 5 mg/m3 of ammonium sulfate aerosol of 0.5 µm MMAD for up to 7 days (23,5h/day).
Furthermore animals were exposed 2 days to ammonium sulfate aerosol alone.

Reliability: (4) not assignable
only one concentration tested, no histological examination, the main examinations refer to ozone, only data of combination exposure are given in this publication, partly insufficient description

10-APR-2006 (131)

Type: Sub-acute
Species: rat **Sex:** male
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 5 or 20 days
Frequency of treatment: 6 hours/day, 5 days/week
Post exposure period: no
Doses: 1 mg/m3 (MMAD 0.4 um, geometric standard deviation 2.2)
Control Group: other: filtered air

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, reagent grade

Remark: In this study, animals with elastase-induced pulmonary emphysema and saline-treated controls were used to determine if lung function changes related to ammonium sulfate exposure were greater (compared with controls) in animals with experimentally induced emphysema. Only the results for ammonium sulfate exposure vs. air controls are reported in detail.

Result: Results from 27-29 animals/group are reported. No information is provided on mortality.
Body weight was not affected by aerosol exposure. In ammonium sulfate exposed rats, values for lung residual volume and functional residual capacity were significantly larger than comparable values measured in air-exposed animals. This

difference between air- and ammonium sulfate exposed animals was both in spontaneously breathing and paralyzed animals. The residual volume / twice total lung capacity (RV/TLC), measured in paralyzed animals, was also significantly higher in ammonium sulfate exposed rats than in air-exposed rats. Carbon monoxide diffusion capacity and quasistatic compliance were not affected by aerosol exposure. Compared with air-exposed animals, ammonium sulfate exposed rats showed a decreased slope of single-breath N2 washout curves.

There were no consistent differences observed between 5- and 20-day exposed animals.

Test condition: The test animals (from Charles River Breeding Labs, Portage, Mich.) were isolated for 3 weeks after delivery. Following isolation, animals were assigned to exposure groups such that preexposure body weight distributions were the same for all experimental groups exposed at the same time.
Anesthetized animals were instilled intratracheally with either sterile saline or porcine pancreatic elastase (EO127, Sigma Chemical Co.; 75 units/100 g bw). Elastase activity was determined immediately before daily instillations by the method of Sachar et al. (1955, Proc. Soc. Exp. Biol. Med. 90, 323-326), using elastin-orcein as substrate.
The doses of elastase were selected as the maximum doses tolerated that produced less than 10% acute mortality, based on results of previous studies (Busch et al., 1984, Environ. Res. 33, 473-496).
Following instillation rats were given prophylactic tetracycline (60 mg/L) in their drinking water for 2 weeks to prevent pulmonary infections, occasionally observed in preliminary studies, following the instillations.
Three weeks following intratracheal instillations, the then approximately 14 week old rats were exposed in Hazleton Systems Model 1000 stainless steel chambers for 5 or 20 days to filtered room air or 1 mg/m3 ammonium aerosol.
Chamber aerosol concentration was monitored continuously and the variations throughout the chamber were less than 5% of the mean concentration. Aerosol concentrations were determined from four daily, 90-min, filter-pad samples; ammonium sulfate concentrations were determined by an automated methyl-thymol blue method. Particle-size distribution was measured from combined Mercer cascade impactor samples, collected over 15 hours of exposure during a 5-day week. Daily impactor samples were collected at a constant flowrate of 2 liters/min on glass coverslips.Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the aerosol were calculated using a program which compared the impactor data with a log-normal distribution (Hill et al., 1979; PNL-2405. Pacific Northwest Laboratory. Richland. WA. Available from NTIS, Springfield.Va.).
For the evaluation of pulmonary function, animals were anesthetized by intraperitoneal injection with ethyl carbamate and then intubated with an esophageal catheter and placed prone in a flow-type plethysmograph. Transpulmonary pressure (airway pressure minus esophageal pressure) was determined by electronic subtraction of the two signals. Before making any measurements, the lungs were inflated twice to total lung capacity to establish a constant volume

history. Lung volumes in spontaneously breathing animals were determined by connecting a syringe to the airway opening, evacuating the lungs to residual volume, then inflating the lungs to total lung capacity. During these maneuvers, volume excursions and transpulmonary pressure were recorded. Residual volume was determined by inert gas dilution, using 0.5% neon in air as the test gas.
Single-breath carbon monoxide diffusion capacity of the lung was determined by a modification of the technique of Takezawa et al (1980, J.App.Physiol. 48, 1052-1059). Following these measurements, animals were paralyzed by intramuscular injections of succinyl choline chloride and artificially ventilated with a rodent respirator. Quasistatic compliance and single-breath N2 washout maneuvers were performed by inflating the lungs with air or O2, respectively at flow rates of 2.5 mL/sec to twice the total lung capacity, and then deflating the lungs, at the same flow rate, to residual volume. Functional residual capacity (the volume of gas in the lungs following normal
passive exhalation) was measured in paralyzed animals using the inert gas dilution technique used to measure residual volumes in spontaneously breathing animals.
Statistical Method: analysis of variance.
Year of study: not reported.

Conclusion: The pattern of lung volume changes observed in ammonium sulfate exposed rats (increased residual volume and functional residual capacity, with no significant change in vital capacity) suggest that ammonium sulfate exposure resulted in mild pulmonary emphysema or slight exacerbation of elastase induced emphysema.

Reliability: (4) not assignable
limited documentation, no further information on purity, only one dose tested.

10-APR-2006 (132)

Type: Sub-acute
Species: rat **Sex:** male
Route of administration: inhalation
Exposure period: 8h/day
Frequency of treatment: 3 days
Doses: 1000-1200 mg/m3, particle size 2-3um

Method: other: see test conditions
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Result: None of the rats died during the exposures. No gross toxicological effects were noted (no further details reported).

Test condition: A group of 6 male rats was exposed(whole body) 8 h/day for 3 consecutive days to a concentration of 1000-1200 mg/m3 (maximum attainable concentration).
Ammonium sulfate aerosol was generated from an aqueous solution with Retec nebulizers and dried by mixing with dry air and passing it through a heated glass tube. Ammonium sulfate concentration was determined by collecting the aerosol on a glass fiber filter at a flow rate of 2 L/min for 15 minutes and weighing the filter. Accuracy of the method was periodically checked by chemical analysis of the

filter sample. Particle size was evaluated gravimetrically using an Andersen multi-stage sampler. The particle size was between 2 and 3 um in diameter.
YEAR OF STUDY: not reported.

Reliability: (4) not assignable
limited documentation, no information on purity and particle size distribution, only one dose tested.

Flag: Critical study for SIDS endpoint
12-APR-2006 (120)

Type: Sub-acute
Species: rat **Sex:**
Strain: Fischer 344
Route of administration: inhalation
Exposure period: 8 weeks
Frequency of treatment: 4 hours/day on 4 consecutive days per week, for a total exposure time of 32 days over an 8-week period
Post exposure period: no
Doses: the tested concentration are given as 20 and 70 ug sulfate/m3 in the introduction, and 20 and 70 ug ammoniumsulfate/m3 in the text of the publication
Control Group: other: purified air

Method: other: see test condition
GLP: no data
Test substance: other TS: ammonium sulfate aerosol, MMAD 0.2 ug, no further details

Remark: F344/N rats (the publication abstract states: Sprague-Dawley)
Result: TOXIC RESPONSE/EFFECT BY DOSE LEVEL:
Mortality, clinical signs: not reported. The treatment had no effect on respiratory frequency and tidal volume during exposure (no further details reported).
Body weight: not reported.
Food/Water consumption: not reported.
Gross pathology incidence and severity: not reported
Organ weight changes: not reported

CLINICAL BIOCHEMISTRY FINDINGS:
No exposure related increases in numbers or percentages of lymphocytes or polymorphonuclear cells were found in the bronchoalveolar lavage fluid (BAL). The treatment had no effect on the release of the inflammatory mediators prostaglandin E2 (PGE2) and leukotriene B4 (LTB4).

Macrophage function:
- 20 µg/m3: macrophages exhibited a significantly depressed ability to attack antigenic material (Sheep Red Blood Cells) via Fc receptor-mediated processes. A trend towards increased production of superoxide by macrophages was observed, but the changes were not significantly different from the control values.
- 70 µg/m3: no statistically significant effect on macrophage function was observed. The production of super-oxide during respiratory burst activity was significantly depressed.

Permeability of the epithelium:

- 20 µg/m3: slightly, but not significantly, elevated albumin level in the bronchoalveolar lavage fluid as indication for an increased permeability of the epithelium

- 70 µg/m3: no effect

HISTOPATHOLOGICAL INCIDENCE AND SEVERITY:
Lung morphometric analysis:
20 µg/m3: not studied
70 µg/m3:
- Alveolar wall nuclear density was increased slightly, but not significantly
- Alveolar septal wall thickness was increased significantly (ca: 20%, only graphic results given)
- Both alveolar chord length (a measure of average alveolar linear. dimension), and alveolar cross-sectional area tended to decrease.
- Significant decrease in alveolar cross-sectional area (ca. 7%, only graphic results given)

The number of goblet cells in the respiratory epithelium was unaffected by the treatment.

No histopathology (light microscopic investigations) was performed or described, respectively, so the cause of the increase in thickness of the alveolar septal wall remains unclear. These results do not account for any kind of emphysema.

No final assessment is possible because of incomplete documentation.

Test condition: The study analyzed the effects of three PM-10 components (ammonium sulfate, ammonium nitrate, and road dust).
ANIMALS: F344/N rats (Simonsen Laboratories, Inc., Gilroy, CA) [note: in the study abstract the use of Sprague-Dawley rats is stated].
Age and Weight at study initiation: not reported.
Number of animals per sex per dose: not reported; 12 animals/group were used for the macrophage function analysis; no information is available on the number of animals and the sex of animals used in the other experiments. For all experiments (i.e. including those performed with road dust and ammonium nitrate), a total of 144 rats were used.

TEST PROCEDURE:
Ammonium sulfate aerosols were generated by nebulization of dilute aqueous solutions. The particles were mixed with dry dilution air, and equilibrated to 60% relative humidity.
Rats were exposed nose-only. The high-dose group was tested in one set of exposures and the low-dose group was tested in a separate set of exposures. Separate purified air control groups were used for high-and low-concentration exposures.
Rats were killed 1-2 h after the end of exposure.

CLINICAL OBSERVATIONS: not reported

EXAMINED PARAMETERS:
Separate groups were assessed for histopathology endpoints and for macrophage and permeability-related end-points.

- Morphometric analyses were performed on lungs of rats exposed to purified air or to the high concentration, and included measurements of the thickness and cellularity of the alveolar wall, alveolar chord lengths and alveolar cross-sectional diameters.
- Macrophage function analysis: Bronchoalveolar lavage (BAL) was performed on 12 rats per group to obtain macrophages for immunological testing and proteins for assessment of epithelial permeability. Changes in functional characteristics of alveolar macrophages were quantified by a rosette assay for determining Fc receptor binding capacity for sheep red blood cells, and by determination of the production of superoxide anion during respiratory burst activity.
- Changes in airway permeability: permeability was determined by measurement of albumin concentrations in the BAL fluid.
- Further exploratory endpoints that were measured were: determination of the numbers of goblet cells in the respiratory epithelium, releases of the inflammatory mediators prostaglandin E2 (PGE2) and leukotriene B4 (LTB4), and measurements of respiratory frequency and tidal volume during exposure.

STATISTICAL METHOD: one-way or two-way analyses of variance, as appropriate. Tukey multiple comparison test for differences between group means. The criterion for statistical significance was p = 0.05.

Reliability: (3) invalid
Insufficient and partly contradictory documentation. The experiments were performed in separate sets of exposures, with separate control groups demonstrating a high variability in results. Morphometric analyses were performed in only one dose-group, and the relevance of the subtle changes reported remains therefore unclear. The findings relating to macrophage function were not dose related, and appear to be within the normal physiological ranges. Unacceptable reporting.

21-JUN-2004 (133)

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: Fischer 344
Route of administration: oral feed
Exposure period: 13 weeks
Frequency of treatment: continuously
Post exposure period: no
Doses: 0, 0.38, 0.75, 1.5, 3% in diet (corresponding to 0, 222, 441, 886, 1792 mg/kg bw/day in males and to 0, 239, 484, 961, 1975 mg/kg bw/day in females).
Control Group: yes, concurrent no treatment
NOAEL: = 886 mg/kg bw
NOAEL, females : = 1975 mg/kg bw

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, purity not stated

Result: BODY WEIGHTS: at study end final body weights were in males 298, 273, 287, 282 and 284 g for the 0, 0.38, 0.75, 1.5 and 3% groups, respectively. In females, final body weights were

151, 157, 152, 161 and 158 g for the 0, 0.38, 0.75, 1.5 and 3% groups, respectively.
FOOD INTAKE (g/rat/day): 14.2, 14.0, 14.3, 14.1, 13.8 in the males of the 0, 0.38, 0.75, 1.5 and 3% groups, respectively. In females, the values were 9.2, 9.1, 9.3, 9.3 and 8.4 for the 0, 0.38, 0.75, 1.5 and 3% groups, respectively.
HEMATOLOGY AND CLINICAL CHEMISTRY PARAMETERS: no biologically significant changes were observed in any of the investigated parameters. Though there were statistical differences in some of the parameters, there was no consistent dose-effect relationship and/or all values were within the normal ranges of values normally found in the rat strain used in this study. In particular, there were no signs indicative of a metabolic acidosis.
ORGAN WEIGHTS: No significant changes in absolute or relative organ weights were observed for brain, lung, heart, spleen, liver, adrenals, kidney and testis weights. Increases (<15%) in the relative and absolute kidney weights in high dose male and females, and in liver weight in high dose females (+11%), were not accompanied by any functional (clinical parameters) or histopathological changes, and were therefore not considered as adverse effects by the authors.
HISTOPATHOLOGICAL EXAMINATIONS: no significant pathological effects were found.
In the 3% male group myofibrosis cordis, basophilic kidney tubulus as well as splenic melanosis were observed; in the female group basophilic kidney tubulus as well as splenic melanosis were observed. However the rate of occurrence was similar to controls.

Test condition: TEST ANIMALS/HOUSING: 5 week old SPF F344/DuCri rats from Charles River Nippon were used and acclimatized for 1 week prior to the start of the experiments (24 +/- 1 degree C, relative humidity 55 +/-5%, 18 air exchanges/day, 12 hour light/dark cycle). Animals were maintained on CRF-1 powder diet. Rats were randomly divided into five groups, each group consisting of 10 males and 10 females.
EXPOSURE TO TEST SUBSTANCE:
The animals were fed CRF-1 powder diet containing concentrations of 0, 0.38, 0.75, 1.5, and 3.0% of ammonium sulfate. The dose levels were set on the basis of results from a previous 2-week study with a dose level of 5% (no further details reported).
ORGANS WEIGHED:
At necropsy, the following organs were weighed and absolute and relative organ weights determined:
males: brain,lung,heart,spleen,liver,adrenal,kidney,testis.
females: brain,lung,heart,spleen,liver,adrenal,kidney.
ORGANS EXAMINED HISTOPATHOLOGICALLY:
males: brain,lung, heart, spleen, liver, adrenal, kidney, testis.
females: brain,lung, heart, spleen, liver, adrenal, kidney.
Organs were fixed in formalin and hematoxylin-eosin slides were prepared and examined histologically.
CINICAL CHEMISTRY, HEMATOLOGY:
The following clinical parameters were determined:
total protein (TP), albumin/globulin (A/G), albumin, total cholesterol, BUN, Na, Cl, K , Ca, P, GOT, GPT, alkaline phosphatase. Hematology included red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hk), mean corpuscular volume

(MCV), mean erythrocyte hemoglobin (MCH), mean erythrocyte hemoglobin concentration (MCHC), platelet count, and leukocyte count.
STATISTICAL METHODS: Bartlett, and Kruskal-wallis tests, and parametric Dunnett and Scheffe tests.
YEAR Of STUDY: not reported.

Conclusion: Male animals in the 3% group exhibited diarrhea during the administration period. No changes indicating obvious ammonium sulfate toxicity were observed in the body weights, organ weights, hematological, serum biochemical, or histopathological examinations.
Based on these results, the NOEL was judged to be 1.5% in males (886 mg/kg bw/day) and 3% in females (1975 mg/kg bw/day).

Reliability: (2) valid with restrictions
low number of organs examined histopathologically as compared to a guideline study

Flag: Critical study for SIDS endpoint

10-APR-2006 (134)

Type: Sub-chronic
Species: mouse **Sex:** male
Strain: Balb/c
Route of administration: drinking water
Exposure period: 1 month
Frequency of treatment: continuously
Post exposure period: no
Doses: 0.611 g/L ammonium sulfate (corresponding to 250 ppm ammonium)
Control Group: yes, concurrent vehicle

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not further specified

Remark: The study was undertaken to investigate the role of cytokines involved in aluminum neurotoxicity. Animals were treated with aluminum ammonium sulfate at different dose levels, and an additional group of animals received ammonium sulfate in order to determine whether ammonium or sulfate ions affected the results.

Result: There were no treatment-related effects on final body weight and on liver, kidney, and spleen weights, normalized to the body weight. No signs of gross behavioral alterations were observed in any animal during the treatment period. There were also no significant differences among controls and treatment groups in weight gain. The food consumption was 4.52 g/mouse per day for the control, and 4.63 g/mouse per day for the ammonium sulfate-treated group. Mean water consumption was 4.574 +/- 0.182 mL/mouse per day for controls, and 4.484 +/- 0.081 mL/mouse per day for ammonium sulfate treated animals.
There were no significant differences for any cytokine between the control and ammonium sulfate treated groups (TNF alpha, IL-1beta, IFN-gamma, and beta-actin in the cerebrum), whereas aluminum treated groups demonstrated a significant increase of relative expression of TNFalpha in the cerebrum.

Test condition: TEST ORGANISMS: adult, 7-8 week old, male BALB/c mice from

Harlan Sprague Dawley (Indianapolis, Ind.). were acclimated for 1 week at 21 °C, 50% humidity, and a 12:12 h light/dark cycle. The mean initial body weight was 23.0 +/- 1.7g. Mice were housed in groups of five, and maintained on commercial rodent chow (Harlan Teklad 22/5 rodent diet, Harlan Teklad, Madison, Wis.).
EXPOSURE TO TEST SUBSTANCE: Mice were administered aluminum ammonium sulfate in deionized drinking water ad lib at levels of 0, 5, 25, and 125 ppm as aluminum for 1 month. Controls received deionized water. An additional group was administered 0.611 g/L of ammonium sulfate to provide an ammonium ion concentration of 250 ppm. The results of this additional group were compared with those of the control to determine whether ammonium or sulfate ions affected the results.
EXAMINATIONS: The weights of mice were recorded weekly and water consumption measured daily. Animals were observed daily for general behavior such as appearance, activity, grooming, and locomotion. On the last day of treatment, brains were isolated and the cerebrum was dissected from brain. A representative sample of whole cerebrum was employed to extract RNA. RNA was quantified by spectrophotometric absorbance at 260 nm. In PCR amplified samples, the following cytokines were determined: TNF-alpha, IL-1beta, and IFN-gamma. beta-actin from cDNA was also amplified and served as internal standard. Splenic macrophages and lymphocytes were collected according to the method previously described by Sharma, 1996. Liver, kidney and spleen were weighed.
STATISTICAL EVALUATION:
The log-transformed data of control and treatment groups were compared by one-way analysis of variance (ANOVA) followed by Fisher`s post-hoc least significant difference (PLSD) test using the Statview software (Abacus Concept, Berkeley, Calif.). For statistical analyses different treatment groups were compared to the group given deionized water.
YEAR of STUDY CONDUCT: not reported.

Reliability: (2) valid with restrictions
limited documentation, no guideline study, no histopathology, only three organ weights determined, only one dose tested

10-APR-2006 (135)

Type: Sub-acute
Species: rabbit **Sex:** female
Strain: no data
Route of administration: oral unspecified
Exposure period: 5-16 months
Frequency of treatment: on alternate days
Post exposure period: no

Method: other: see Test Condition
GLP: no
Test substance: other TS: ammonium sulfate, not further specified

Result: At necropsy, mean body weights were between 4,100 and 4,800 grams. Parathyroid weights were between 15 and 33 mg (mean value 27.5 mg). Parathyroids of control animals (n=50)

weighed between 6 and 14 mg (mean value 9.2 mg). Similar or higher increases in parathyroid weight were also found with all other chemicals tested in this study (ammonium hydroxide, carbonate, chloride, hydrophosphate, acetate, lactate, calcium chloride, hydrochloric acid, lactic and acetic acids, sodium ammonium phosphate, and sodium dihydrogen phosphate). The author considers this effect to be caused by metabolic acidosis that was induced by the various chemicals (though no experimental data relating to the latter were provided in the publication).

Test condition: 10 females were treated with 100-200 mg ammonium sulfate / kg bw, dissolved in 100-150 cm3 drinking water every second day. The dose was gradually increased over the experimental period of 5-16 months (no further details given). Each 4th week was scheduled as a recovery period without treatment.

Reliability: (4) not assignable
documentation insufficient for assessment

19-JUL-2004 (136)

Type: Sub-chronic
Species: Syrian hamster **Sex:** male
Route of administration: inhalation
Exposure period: 15 weeks
Frequency of treatment: 6 hours/day, 5 days/week
Doses: 186.6 ug/m3 (analytical) (200 ug/m3 , nominal)
Control Group: other: BaP

Method: other: see Test Condition
Year: 1978
GLP: no
Test substance: other TS: ammonium sulfate, no further specified

Remark: The study evaluated the influence of ammonium sulfate as a cofactor in carcinogenesis studies employing benzo(a)pyrene as the prime agent. Additional information concerning influence on a metabolic enzyme (arylhydroxylase) and the body`s absorption and excretion of sulfate was also included (cf IUCLID section on toxicokinetics). NOTE: wrong units (mg instead of ug) were used in some parts of the publication by Godleski et al., 1984.

Result: Inhaled ammonium sulfate did not increase the incidence or severity of pneumonitis or pulmonary fibrosis in the hamster. This inhalation did increase the incidence of emphysema as examined microscopically but not the severity. The increase in emphysema incidence was not statistically significant (9.0 vs 15.2%, EPA 1978). Another publication of this study described an increase in emphysema incidence, which is however of doubtful statistical significance. It was based on only 80 animals per group (8.6 vs 16.1%, Godleski, 1984). Due to the higher number of animals used the EPA results are more reliable and 0.2 mg/ammonium sulfate/m3 cannot be regarded as a LOAEL. However it seems not acceptable to take 0.2 mg/m3 as the NOAEL from this study because the animals were not examined immediately after exposure but only after 2 years.

Test condition: 80 approximately 10-week old animals were exposed for 6 hours/day, 5 days/week for 15 weeks at an average concentration of 186.6 ug/m3 (nominal concentration 200 ug/m3).
After 2 years, histological sections of the respiratory tract

(nose, larynx, bronchii, the lobes of the lung) were prepared, and hematoxylin- and eosin- stained slides were evaluated. The study was repeated and the results of both studies with 160 animals was reported in the EPA report (1978) and with 80 animals in the literature of Godleski. (1984).

Reliability: (4) not assignable
The interval between termination of exposure and examination was too long, therefore the reliability is 4

Flag: Critical study for SIDS endpoint

12-APR-2006 (104) (137)

Type: Sub-acute
Species: guinea pig **Sex:** male
Strain: Hartley
Route of administration: inhalation
Exposure period: 4 weeks
Frequency of treatment: 6 hours/day, 5 days/week
Post exposure period: no
Doses: 1.03 mg/m3; MMAD 0.42 um
Control Group: other: sham exposure

Remark: According to the study authors, the described alterations in the secretory response may have protected the lungs of exposed animals from the adverse effects of inhaled aerosols and may have contributed to the functional differences observed between ammonium sulfate exposed rats (see study Busch et al. 1984) and guinea pigs.

Result: An apparent alteration in secretory activity characterized by hypertrophy and hyperplasia of nonciliated epithelial cells, with an increased number of secretory granules per cell was observed in the lungs of animals exposed to ammonium sulfate aerosol. These changes were seen in airways ranging from small bronchi to terminal respiratory bronchioles.

Test condition: Young adult animals (Charles River Lab., Kingston, NY) were maintained in isolation for 3 weeks prior to beginning experimental procedures. Animals were assigned to four experimental groups based on their initial weights, so that the distribution of animal weights for each treatment group was the same. The animals were divided into two groups, one to receive intratracheally instilled porcine pancreatic elastase ("elastase impaired") and one to receive saline solution intratracheally (controls). 30 guinea pigs received 5 units of elastase activity / 100 g bw. The dose level was determined in preliminary experiments to produce a predictable degree of emphysema without inducing significant mortality. No data about number of saline treated guinea pigs are evaluable.
Sterile elastase or saline solutions, standardized for activity (in the case of elastase), osmolarity, pH, and temperature, were administered intratracheally to ether-anesthetized animals. The total volume of the instillate varied slightly, depending on animal weight; however, concentration/volume was constant. Following recovery from anesthesia, the animals were caged for a 3-week recovery period.
EXPOSURE TO TEST SUBSTANCE:
One-half of the elastase-treated animals and one-half of the

saline-treated animals were exposed to ammonium sulfate, and the remainder were exposed to air only. Animals were exposed and housed in Hazelton Systems Model 100 stainless-steel exposure chambers. The chamber air flow of 0.30 m3/min provided seven changes of filtered air per hour. Aerosols were introduced into and thoroughly mixed with the filtered air stream before entering the chambers. Several Retec nebulizer generators supplied with special reservoirs provided a constant ammonium sulfate solution concentration and liquid level for stable aerosol generation.
Time-weighted average concentrations of aerosol were determined from four 90-mm filter samples taken over the daily 6-hr exposure periods. These filter samples were analysed, chemically and gravimetrically, and corrected by correlating the amount of particles recovered by spiked filters with each set of samples. Daily
time-weighted-average concentrations for ammonium sulfate were 1.03 mg/m3 +/- 0.11 SD (gravimetric) and 0.97 mg/m3 +/-0.11 SD (chemical). Aerosol particle size was measured by a cascade impactor (Mercer design) on samples collected over 15 hours of exposure time during a 5-day week. The MMAD was 0.42 +/- 0.05 um SD, geometric standard deviation (GSD) was 2.25 +/- 0.22.
Animals were exposed for 6 hours/day, 5 days/week, for a total of 20 exposures. Control (air only) animals were maintained in identical chambers under similar conditions of air flow, temperature, humidity, cage changes, etc. All animals were housed in similar chambers during nonexposure periods.
At the end of the exposure period (4 weeks after intratracheal instillations), animals were deeply anesthetized, and the lungs perfused with 2% glutaraldehyde solution. The lungs were removed for examination after observation of macroscopic features in situ. Fixed lung volume was determined by weight of displaced fluid.
Slices of approximately 1 mm were taken from both the right diaphragmatic and left lobes of each animal and examined under a dissecting microscope for the degree of emphysema. A subjective rating of 0 (none observed) to 5 (total involvement) was given to each tissue slice. The rating (score) was determined by estimating the proportion of the slice affected by disruptive alveolar changes and the severity of alveolar disruption within involved areas. The area affected was determined.
From each animal, the slice most closely approximating the mean degree of involvement was prepared for examination by scanning electron microscopy (SEM).
Morphometry: chord lengths were measured across alveoli, alveolar sacs, and alveolar ducts from photographs taken during SEM. The median, mean, and SD were calculated for these chord length measurements, and histograms were developed. To ensure consistency in measurements between photographs the criteria as developed by Busch et al., 1984 (Env. Res. 33, 497-513) were followed. In addition, alveolar pores, the number of
nonciliated bronchiolar epithelial cells per standardized area was counted, and means and SD were calculated.
A section of the right cardiac lobe was prepared for examination by light microscopy to identify any pathologic

processes.
Statistical analysis: two-way analysis of variance.
Reliability: (4) not assignable
no information on purity
10-APR-2006 (128) (129)

Type: Sub-acute
Species: guinea pig **Sex:** male
Strain: Hartley
Route of administration: inhalation
Exposure period: 5 or 20 days
Frequency of treatment: 6 hours/day, 5 days/week
Post exposure period: no
Doses: 1 mg/m3 (MMAD 0.4 um, geometric standard deviation 2.2)
Control Group: other: filtered air

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, reagent grade

Remark: In this study, animals with elastase-induced pulmonary emphysema and saline-treated controls were used to determine if lung function changes related to ammonium sulfate exposure were greater (compared with controls) in animals with experimentally induced emphysema.

Result: Results from 29 treated and 27 control animals are reported. No information is provided on mortality.
Body weight was not affected by ammonium sulfate exposure or elastase treatment. Ammonium sulfate exposures had no significant effect on any of the measured lung volumes, carbon monoxide diffusion capacity or on quasistatic compliance. There were no consistent differences observed between 5- and 20-day exposed animals.

Test condition: The test animals (from Charles River Breeding Labs, Kingston, Mass.) were isolated for 3 weeks after delivery. Following isolation, animals were assigned to exposure groups such that preexposure body weight distributions were the same for all experimental groups exposed at the same time.
Anesthetized Animals were instilled intratracheally with either sterile saline or porcine pancreatic elastase (EO127, Sigma Chemical Co.; 5 units/100 g bw). Elastase activity was determined immediately before daily instillations by the method of Sachar et al. (1955, Proc. Soc. Exp. Biol. Med. 90, 323-326), using elastin-orcein as
substrate. The doses of elastase were selected as the maximum doses tolerated that produced less than 10% acute mortality, based on results of previous studies (Busch et al., 1984, Environ. Res. 33, 473-496).
Three weeks following intratracheal instillations, the then approximately 10 week old guinea pigs were exposed in Hazleton Systems Model 1000 stainless steel chambers for 5 or 20 days to filtered room air, 1 mg/m3 ammonium sulfate or 1 mg/m3 ammonium nitrate aerosols.
Chamber aerosol concentration was monitored continuously and the variations throughout the chamber were less than 5% of the mean concentration. Aerosol concentrations were determined from four daily, 90-min, filter-pad samples; ammonium sulfate concentrations were determined by an

automated methyl-thymol blue method. Particle-size distribution was measured from combined Mercer cascade impactor samples, collected over 15 hours of exposure during a 5-day week. Daily impactor samples were collected at a constant flowrate of 2 liters/min on glass coverslips. Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the aerosol were calculated using a program which compared the impactor data with a log-normal distribution.
For the evaluation of pulmonary function, animals were anesthetized by intraperitoneal injection with ethyl carbamate and then intubated with an esophageal catheter and placed prone in a flow-type plethysmograph. Transpulmonary pressure (airway pressure minus esophageal pressure) was determined by electronic subtraction of the two signals. Before making any measurements, the lungs were inflated twice to total lung capacity to establish a constant volume history. Lung volumes in spontaneously breathing animals were determined by connecting a syringe to the airway opening, evacuating the lungs to residual volume, then inflating the lungs to total lung capacity. During these maneuvers, volume excursions and transpulmonary pressure were recorded. Residual volume was determined by inert gas dilution, using 0.5% neon in air as the test gas.
Single-breath carbon monoxide diffusion capacity of the lung was determined by a modification of the technique of Takezawa et al. (1980, J.App.Physiol. 48, 1052-1059).
Following these measurements, animals were paralyzed by intramuscular injections of succinyl choline chloride and artificially ventilated with a rodent respirator. Quasistatic compliance and single-breath N2 washout maneuvers were performed by inflating the lungs with air or O2, respectively at flow rates of 2.5 mL/sec to twice the total lung capacity, and then deflating the lungs, at the same flow rate, to residual volume. Functional residual capacity (the volume of gas in the lungs following normal passive exhalation) was measured in paralyzed animals using the inert gas dilution technique used to measure residual volumes in spontaneously breathing animals.
Statistical Method: analysis of variance.
Year of study: not reported.

Reliability: (4) not assignable
limited documentation, no further characterization of purity

10-APR-2006 (132)

Species: other: donkey **Sex:**
Route of administration: inhalation
Exposure period: about a year
Frequency of treatment: 1 hour weekly
Post exposure period: no data
Doses: 0.3 - 3.1 mg/m3 (MAD 0.3-0.6 um)
Control Group: other: H2SO4 aerosol

Method: other: see Test Condition
GLP: no
Test substance: other TS: (NH4)2SO4, purity not stated

Result: Short -term slowing of clearance followed certain single exposures to H2SO4 at 194-1364 ug/m3 (in three of four

animals, while two of the four demonstrated a more persistent clearance. These exposure levels produced no measurable change in resistance, compliance or regional deposition. Exposure to (NH4)SO4 up to ca. 2000 ug/m3 had no measurable effect upon resistance, compliance, regional deposition or mucocilary clearance.

Test condition: The effects of one-hour inhalation exposure 0.3-0.6um H2SO4 and (NH4)2SO4 aerosols were studied in terms of alterations in pulmonary flow resistance and dynamic compliance, and changes in the regional deposition and tracheobronchial mucociliary clearance.
respiratory mechanics
4 Donkeys were exposed with a catheter to H2SO4 aerosol (0.07-1.4 mg/m3) or to ammonium sulfate aerosols (0.3-3.1 mg/m3) for 1 hour/week for about 1 year.
The median aerodynamic diameter (MAD) for both aerosols was 0.3 to 0.6 um and the standard geometric deviation 1.5 um.
prior the sulfate exposure, baseline control values for pulmonary resistance (R1) and dynamic compliance (Cdyn) were obtained by talking at least three successive sets of ten measurements, this allowed determination of spontaneous variability of individual measurements.
The first post-exposure measurements were obtained within one minute of the end of the sulfate aerosol exposure period.
To determine any effects upon mechanics due solely to breathing from the sulfate generation unit, R1 and Cdyn were measured following five sham exposures of each donkey. These sham exposures had no effect upon R1 or Cdyn. regional deposition and clearance.
A series of eleven control tests was performed on each animal to determine baseline values for percentage 24 h-clearance, bronchial clearance half-time, and tracheal transport rate.
Additional control tests were performed on the day preceding a sulfate exposure, and occasionally on the day following.
To determine the effect of sulfate exposure upon regional deposition, each donkey was exposed to sulfate followed, within a minute, by inhalation of the radioactive tagged ferric oxide aerosol.
The exposure were also performed in which ferric oxide was inhaled prior to the one-hour exposure to sulfate.
Three sham exposure controls using each of the above two protocols were performed on each donkey, these had no effect upon regional deposition or clearance.

Conclusion: Exposure to up to 3.1 mg/m3 (1 hour/week, 1 year) had no effect on pulmonary defense mechanisms in donkeys.

Reliability: (3) invalid
no common test system

10-APR-2006 (123)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium TA1535 TA100 TA1537 TA98
Concentration: 0, 20, 100, 500, 2500 and 5000 ug/plate
Cytotoxic Concentration: not cytotoxic up to and including the highest tested concentration level
Metabolic activation: with and without
Result: negative

Method: other: OECD TG 471 (1983)
Year: 1989
GLP: no
Test substance: other TS: as prescribed by 1.1 - 1.4, purity 99.5%

Result: In none of the experiments was an increase in the mutation frequency observed. Cytotoxic effects were not found. The positive controls were functional and showed a distinct increase in mutation frequencies.
Test condition: A standard plate test and a pre-incubation test were performed within this study, both with and without a metabolic activation system (liver S-9 mix from Aroclor 1254 induced male rats).
Solvent: distilled water
Negative controls: solvent control, sterility control.
Positive controls:
- 2-aminoanthracene (10 ug, dissolved in DMSO), for experiments with S-9 mix in TA100, TA98, TA1537 and TA1535
- N-methyl-N´-nitro-N-nitroso-guanidine (MNNG, 5 ug dissolved in DMSO), for experiments without S-9 mix in TA100 and TA1535
- 4-nitro-o-phenylendiamine (10 ug, dissolved in DMSO) for the experiments without S-9 mix in TA98
- 9-aminoacridine chloride monohydrate (100 ug, dissolved in DMSO) for the experiments without S-9 mix in TA1537.
Evaluation criteria:
A substance was considered positive in this test if the following was fulfilled:
- doubling of the spontaneous mutation rate (control)
- dose-response relationship
- reproducibility of the results.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
18-NOV-2003 (138)

Type: Ames test
System of testing: Salmonella typhimurium TA1535 TA1537 TA1538
Concentration: 25,000 and 50,000 ppm (2.5, 5%; 25, 50 mg/mL) for suspension tests; 50,000 ppm (5%; 50 mg/mL) in plate test
Cytotoxic Concentration: not cytotoxic
Metabolic activation: with and without
Result: negative

Method: other: standard plate and suspension tests
Year: 1975
GLP: no data
Test substance: other TS: ammonium sulfate granular food grade

Remark: all positive controls were functional.
Test condition: A standard plate test and a suspension test were performed within this study, both with and without a metabolic activation system (liver S-9 mix from male rats; additionally lung and testes S-9 supernatants from male rats, ICR mice and rhesus monkey were used as well as liver S-9 from mice and monkeys for the activation experiments).
Solvent: saline.
Negative controls: solvent control.

Positive controls:
- 2-acetylaminofluorene and dimethylnitrosamine for experiments with S-9 mix
- ethyl methanesulfonate, 2-nitrofluorene, and quinacrine mustard for experiments without metabolic activation.
Evaluation criteria: not reported.

Reliability: (2) valid with restrictions
limited documentation, no further information on purity

Flag: Critical study for SIDS endpoint

10-APR-2006 (139)

Type: Yeast gene mutation assay
System of testing: Saccharomyces cerevisiae D4
Concentration: 25,000 and 50,000 ppm (2.5 and 5%; 25, 50 mg/mL)
Cytotoxic Concentration: not cytotoxic
Metabolic activation: with and without
Result: negative

Method: other: standard suspension test.
Year: 1975
GLP: no
Test substance: other TS: ammonium sulfate granular food grade

Remark: The ade locus appeared not to respond to the positive control. No reason for the lack of response was evident. The try locus appeared normal and the data was considered to be acceptable.

Test condition: Solvent: Saline.
The tissue homogenates and 9,000 g supernatants were prepared from tissues of the following species: mouse, ICR random bred adult males; rat, Sprague-Dawley adult males; primate, Macaca mulatta, adult males.
positive controls:
activation assay:
Dimethylnitrosamine, 2-acetylaminofluorene
nonactivation assay:
ethyl methanesulfonate
2-nitrofluorene
quinacrine mustard

Reliability: (2) valid with restrictions
limited documentation, no further information on purity

Flag: Critical study for SIDS endpoint

05-NOV-2003 (139)

Type: Cytogenetic assay
System of testing: human lymphocytes
Concentration: 3.2 M (423 mg/mL)
Cytotoxic Concentration: cytotoxicity not reported
Metabolic activation: without
Result: negative

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, analytical grade

Result: Effects of the restriction endonuclease Alu I on chromosomes were more pronounced when the cells were treated additionally with ammonium sulfate.

The chromosomal aberration rates in cells treated with ammonium sulfate alone were not increased:

% ABERRANT METAPHASES (200 metaphases analyzed, including achromatic lesions):
5.5, 6, 6, 3, 3 for controls without treatment, with 20 h buffer treatment, with buffer plus ammonium sulfate treatment (20 h), with 46 h buffer treatment, and with buffer plus ammonium sulfate treatment (46h), respectively.

Test condition: Lymphocytes were isolated from whole blood in a Ficoll gradient. 1x10e6 lymphocytes were incubated in 5 mL plastic tubes in 2.5 mL McCoy`s 5A medium supplemented with 20% fetal calf serum, 0.06 mL phytohemagglutinin M, 100 U/mL penicillin and 0.1 mg/mL dihydrostreptomycin sulfate. The cultures were incubated for 50 hrs, including a treatment with colcemid (0.08 ug/mL) for 3 hrs. Treatment for 15 min with Alu I, ammonium sulfate or Alu I shipping buffer was done at culture times of 20 and 46 hrs, principally as described previously (Obe and Winkel, 1985, Mut Res. 152, 25-29). Shipping buffer consisted of: KPO4, 20 mM; KCl, 50 mM; EDTA, 0.1 mM; dithioerythritol, 10 mM; glycerin, 50% (v/v), pH 7.5. Up to 7 independent experiments were performed with blood from 5 different donors. Preparations were made following a routine protocol and were stained with Giemsa stain.
The following aberrations were analysed: achromatic lesions, chromatid breaks, isochromatid/chromosome breaks, chromatid intrachanges, chromatid interchanges, triradials, polycentric chromosomes, ring chromosomes and minutes.
YEAR OF STUDY: not reported.

Reliability: (2) valid with restrictions
limited documentation; only one dose level, no standard test system for chromosomal aberrations

Flag: Critical study for SIDS endpoint

07-NOV-2003 (140)

Type: Cytogenetic assay
System of testing: CHO cells
Concentration: 0; 0.8; 1.6; 3.2 M (0; 106; 211; 423 mg/mL)
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: negative

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not specified further

Result: 3.2M Ammonium sulfate did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cells. However, high concentrations of ammonium sulfate, magnesium chloride, calcium chloride or sodium chloride increased the frequency of chromosome-type aberrations in CHO cells induced by the restriction endonuclease Alu 1.

The results for cultures treated with Alu I, Alu I + ammonium sulfate and control cultures were as follows:

Treatment / Percent aberrant metaphases / DIC*
Alu I / 40.7 / 34.3

Alu I + 0.8M (NH4)2SO4 / 59.0 / 22.0
Alu I + 1.6M (NH4)2SO4 / 76.5 / 42.5
Alu I + 3.2M (NH4)2SO4 / 98.2 / 240.3
control (Alu buffer) / 16.1 / 4.1
Alu buffer + 3.2 M (NH4)SO4 / 22.5 / 6.0

*DIC: Percent aberrant metaphases, including achromatic lesions and dicentric chromosomes per 100 metaphases as calculated from all polycentric chromosomes induced in CHO cells by 24 units Alu I in the presence or absence of salts. Four possibilities for this effect were discussed by the authors: (1) salt enhances the permeability of the cell membrane, (2) salt changes the structure of the chromatin, (3) salt changes the structure of the DNA, (4) salt influences the repair of damaged DNA.

Test condition: Pellets of 4 x 10e6 cells were made from cultures grown for 2 days and washed once with 1 mL newborn calf serum (NCS). The pellets received 24 units Alu I (Boehringer Mannheim) in a volume of 8 µl containing Alu I (3.4 or 4 µL) and NCS. After mixing, the cells were incubated for 20 minutes at 37 °C in the incubator. Ammonium sulfate was dissolved in distilled water and 10 µL were added 5 minutes after the addition of the AluI. The cells were mixed and reincubated for a further 15 minutes. Respective controls were set up in the same way, but instead of Alu I the cells were treated with the buffer in which the Alu I was shipped.
After the treatment the cells of each pellet were seeded in 2 6-cm petri dishes and incubated in medium containing fetal calf serum instead of NCS (10%), and bromodeoxyuridine (BrdUrd) in a final concentration of 2 x 10e-5 M.
Preparations were made 18 hours later including a 2-hour treatment with Colcemid. The chromosomes were differentially stained following the method of Hill and Wolff (1982, Cancer Res. 42, 893) and exclusively first post-treatment metaphases were analyzed with respect to chromosomal aberrations. The number of dicentric chromosomes (DIC) per 100 metaphases were calculated from all polycentric chromosomes in such a way that from the polycentric chromosomes with more than 2 centromeres, one centromere was subtracted and the remaining number of centromeres was taken as the number of DIC.

Number of metaphases analyzed / number of independent experiments:
Alu I: 1100 / 11
Alu I + 0.8M ammonium sulfate: 200 / 2
Alu I + 1.6M ammonium sulfate: 200 / 2
Alu I + 3.2M ammonium sulfate: 600 / 6
Alu buffer (control): 1100 / 11
Alu buffer + 3.2M ammonium sulfate: 200 / 2

Year of study: not reported.

Reliability: (2) valid with restrictions
limited documentation, no information on purity, no standard chromosome aberration test

Flag: Critical study for SIDS endpoint

12-APR-2006 (141)

Type: Cytogenetic assay
System of testing: CHO cells

Concentration: 3.2 M (423 mg/mL)
Cytotoxic Concentration: cytotoxicity not reported
Metabolic activation: without
Result: negative

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, analytical grade

Remark: The authors suggest that ammonium sulfate may induce conformational changes in the chromatin which make more recognition sites available for the Alu I enzyme.
The frequencies of chromosomal aberrations induced by the restriction endonuclease Alu I (recognition site AG/CT) could be elevated to a similar extent by additional treatments with a single-strand-specific endonuclease from Neurospora crassa, or with ammonium sulfate in which the Neurospora endonuclease was suspended.
Result: Effects of the restriction endonuclease Alu I on chromosomes were more pronounced when the cells were treated additionally with ammonium sulfate. The chromosomal aberration rates in cells treated with ammonium sulfate alone were not increased:

% ABERRANT METAPHASES (200 metaphases analyzed, 22 hr after the treatment with 3.2 M ammonium sulfate for 15 min; including achromatic lesions): 15.5% (control with Neurospora crassa endonuclease instead of ammonium sulfate: 20%).
Test condition: Chinese hamster ovary cells (CHO) were grown in McCoy`s 5A medium, supplemented with 10% fetal calf serum, 100 units penicillin and 0.128 mg/mL dihydrostreptomycin sulfate.
4x10e6 cells from exponentially growing cultures were centrifuged and repelleted in 1 mL newborn calf serum (NCS). Alu I was prepared by mixing stock solution with NCS to give a final volume of 8 uL which was added to the pellet. Neurospora endonuclease or 3.2 M ammonium sulfate solution in distilled water were added to the Alu I-treated cells at different times. The treatments were finished 20 minutes after addition to Alu I by washing the cells.
Controls included cells treated with medium + NCS (8 uL) and 5 minutes later with 10 uL 3.2 M ammonium sulfate or neurospora endonuclease, added for 15 minutes. Cells were fixed and stained 22 hours after treatment, including a treatment with colcemid (0.08 ug/mL) for 2 hrs.
The following aberrations were analysed: achromatic lesions, chromatid breaks, isochromatid/chromosome breaks, chromatid intrachanges, chromatid interchanges, triradials, polycentric chromosomes, ring chromosomes and minutes.
YEAR OF STUDY: not reported.
Reliability: (2) valid with restrictions
limited documentation; only one dose level studied, no standard chromosome aberration test, no negative control, however a very high concentration has been used.
10-APR-2006 (142)

Type: Cytogenetic assay
System of testing: V79 cells
Concentration: osmotic solution of 300 mOsm/kg H20, and hypotonic salt

solutions (200, 150, 75 mOsm); corresponding to about 100; 66.6; 50; 25 mM ammonium sulfate (= 13.2; 8.8; 6.6; 3.3 mg/mL)
Cytotoxic Concentration: treatment with less than 30 mOsm/kg H20 was cytotoxic
Metabolic activation: without

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, purity 99.5%

Remark: The study was designed to investigate the effect of hypotonic solution on the induction of chromosomal aberrations.
The reported value of 7.5 % aberrant cells for the 300 mOsm dose level fell well within the laboratory´s historical control range and was hence considered a negative result (personal communication).

Result: Hypotonic solutions of the culture medium alone or from chemicals including ammonium sulfate revealed an increase of chromosomal aberrations. Ammonium sulfate mostly induced chromatid type aberrations. Treatment with less than 30 mOsm/kg H2O was cytotoxic. Reasons for the aberrations observed in hypotonic media may be a directly induced DNA damage such as double strand breaks or a release of DNAse after lysosomal damage because of the hypotonic treatment. Other reasons involved in the induction of aberration production may be changes of the internal pH or damage of the chromosomal proteins.

% ABERRANT METAPHASES (100-300 metaphases analyzed, fixation time 15 hours):
1.0 %, 7.5%, 19.0%, 36.6% and 60.3% for controls, 300, 200, 150 and 75 mOsm ammonium sulfate, respectively.

Test condition: YEAR OF STUDY: not reported.
TEST SYSTEM: V79 hamster cells were grown at 37 deg C in a humidified atmosphere with 5% CO2 in McCoy`s 5A medium, supplemented with 10% fetal calf serum, 100 units penicillin, and 0.128 mg/mL dihydrostreptomycin sulfate in 10-cm petri dishes; 48 hr before the treatment 10e5 cells from the stock culture were seeded in 6-cm petri dishes with 5 mL of complete medium.

PREPARATION OF SALT SOLUTIONS AND MEDIUM: Ammonium sulfate, sodium chloride and trishydroxymethylaminomethane, were dissolved in distilled water; the stock solution was further diluted until the appropriate osmolality was reached. Undiluted McCoy`s medium had 300 mOsm/kg H2O. A dilution of 1:9 (medium:water) reduced the osmolality to 30 mOsm/kg H20. For ammonium sulfate, sodium chloride, and trishydroxymethyl aminomethane there is a clear correlation of molarity and osmolality. The
molarity (mM) of ammonium sulfate solutions has to be multiplied by a factor of 3 to give the osmolality (mOsm/kg H20). The osmolality of all solutions was measured with a microosmometer.
The pH of the ammonium sulfate and sodium chloride solutions was 5.5 independent of the concentration. The pH of McCoy´s medium changed with dilution. Undiluted medium had a pH of

7.2; a dilution to 200 mOsm/kg H2O had no influence on pH. Further dilution led to a pH decrease and medium of 55 mOsm/kg H2O had a pH of 5.9.
HYPOTONIC TREATMENT: Hypotonic treatment was carried out on monolayer cells in petri dishes. The culture medium was discarded, then the cells were washed with 5 mL of prewarmed phosphate-buffered saline (PBS). Immediately afterwards, 5 mL of prewarmed salt solution or diluted McCoy´s medium was added. After 30 minutes at 37 degr C, the salt solution or the hypotonic medium was discarded, the cells were washed once with PBS, and then freshly prepared culture medium with BrdUrd was added. After a culture time of 16 hr, including a 2-hr colcemid treatment, the cells were fixed in methanol:acetic acid and stained with 5% Giemsa or according to the fluorescence plus Giemsa (FPG) technique. All experiments were performed twice or three times without BrdUrd and once with BrdUrd labeled cells.

Reliability: (3) invalid
Unsuitable test system (mostly unphysiological, hypotonic test conditions; the isotonic exposure exceeded the maximum recommended exposure level of 5 mg/mL).

12-APR-2006 (143)

System of testing: E. coli
Result: positive

Reliability: (3) invalid
Erroneously cited. Original publication (Demerec et al., 1951) does not contain data relating to ammonium sulfate.

18-APR-2003 (11)

Type: other: DNA synthesis
System of testing: nucleotides, nucleic acid synthetic analogue and DNA polymerase from avian myeloblastosis virus
Concentration: at least 7 concentrations between 20 um and 150 uM (not specified)
Cytotoxic Concentration: not reported
Metabolic activation: without
Result: negative

Method: other: see Test Condition
GLP: no data
Test substance: other TS: ammonium sulfate, not specified further

Result: In a screening experiment 31 salts were tested for their ability to affect the accuracy of DNA synthesis in vitro. A good correlation was found between known metal carcinogens and the decrease in accuracy of DNA synthesis. Ammonium sulfate had no effect on the precision of DNA synthesis in this test system.
Test condition: The influence of various salts on the accuracy of DNA synthesis was investigated in a solution of nucleotides containing a nucleic acid synthetic analogue. As matrix virus DNA polymerase and 31 different salts were used.
Reliability: (4) not assignable
insufficient information available to allow assessment of reliability

19-MAY-2003 (144)

Type: Cytogenetic assay
System of testing: V79 cells
Concentration: hypertonic solutions with final osmolalities of 500, 750, 1000 or 1500 mOsm/kg H2O (corresponding to about 166.6; 250; 333.3; 500 mM = 22; 33; 44; 66 mg/mL) (converted according to information given by Nowak C (1987), Ter Carc Mut 7, 515)
Cytotoxic Concentration: not cytotoxic up to and including the highest tested osmolality (1500 mOsm)
Metabolic activation: without

Method: other
GLP: no data
Test substance: other TS: ammonium sulfate, purity 99.5%

Remark: The study was designed to investigate the effect of hypertonic solutions on ethylmethane sulfonate induced HPRT mutations and chromosomal aberrations.
Result: Treatment of ethyl methanesulfonate-induced cells with hypertonic solutions containing sodium chloride or ammonium sulfate had a clear effect on chromosomal aberration but TGr (6-thioguanine resistance) mutations were only enhanced by ammonium sulfate posttreatment, and not by sodium chloride posttreatment.

% ABERRANT METAPHASES (200 metaphases analyzed, 2 independent experiments, fixation time 13 hours):
1.0 %, 1.0%, 2.0%, 4.5% and 8.0% for controls, 500, 750, 1000 and 1500 mOsm ammonium sulfate, respectively.
8.0%, 42.0%, 42.5%, 51% and 43% for EMS, EMS+500, EMS+750, EMS+1000 and EMS+1500 mOsm ammonium sulfate, respectively.

% ABERRANT METAPHASES (200 metaphases analyzed, 2 independent experiments, fixation time 16 hours):
1.0 %, 3.5%, 3.5%, 3.0% and 7.5% for controls, 500, 750, 1000 and 1500 mOsm ammonium sulfate, respectively.
8.0%, 8.5%, 19.5%, 30.5% and 26.5% for EMS, EMS+500, EMS+750, EMS+1000 and EMS+1500 mOsm ammonium sulfate, respectively.

TG MUTATIONS (per 10e6 survivors, 2 independent experiments:
28.0 %, 48.7%, 55.95%, 49.9% and 65.5% for controls, 500, 750, 1000 and 1500 mOsm ammonium sulfate, respectively.
314.6%, 584.0%, 512.4%, 478.0% and 782.4% for EMS, EMS+500, EMS+750, EMS+1000 and EMS+1500 mOsm ammonium sulfate, respectively.

It was suggested by the author that hypertonic salt posttreatment leads to conformational changes in the DNA, resulting in an increase in TGr mutations and chromosomal aberrations.
Test condition: YEAR OF STUDY: not reported.
TEST SYSTEM: V79 hamster cells were grown at 37 deg C in a humidified atmosphere with 5% CO2 in Earle`s minimal essential medium (MEM), supplemented with 10% fetal calf serum, 100 units penicillin, and 0.128 mg/mL

dihydrostreptomycin sulfate in 10-cm petri dishes; 48 hr before the treatment 2x10e5 cells from the stock culture were seeded in 10-cm petri dishes with 10 mL of complete medium.
MUTAGEN TREATMENT: Before adding the mutagen the culture medium was discarded and replaced by 10 mL of prewarmed MEM. 2 mL of ethyl methanesulfonate (EMS), dissolved in MEM were added to each dish, to give a final concentration of 20 mM. After 1 h of treatment at 37 deg C, the medium was removed and the dishes were washed twice with 10 mL of prewarmed phosphate-buffered saline (PBS).
POST-TREATMENT WITH AMMONIUM SULFATE or SODIUM CHLORIDE:
The hypertonic treatment was performed immediately after the mutagen treatment. The washing solution was carefully removed, then the cells were covered with 300 uL of PBS to avoid drying. Then 300 uL of ammonium sulfate or sodium chloride solution were added to give a final osmolality of 500, 750, 1000 or 1500 mOsm/kg H2O. Controls and EMS-treated cultures received 600 uL of PBS (300 mOsm/kg H2O). The osmolality of all solutions was measured with a microosmometer. After 30 min at 37 deg C the treatment was stopped by adding 5 mL of PBS. The supernatant of all dishes was collected in centrifuge tubes so that no cells were lost. Then the cells were trypsinized by adding 2 mL of trypsin/EDTA. After 3 min the cells were collected with 2 mL of medium and added to the supernatant. After centrifugation the cells were counted and plated for the chromosomal aberration and TGr mutation assay.
CHROMOSOMAL ABERRATIONS:
Immediately after the posttreatment with hypertonic salt solutions 10e6 cells were transferred to 6-cm dishes and cultivated for 13 or 16 hours, respectively, in complete medium at 37 deg C, including a 2.5 h colcemid treatment. The cells were fixed in methanol-acetic acid and stained with 5% Giemsa. In some experiments cultures containing BrdUrd were set up in parallel to ensure that only first post-treatment metaphases were scored for chromosomal aberrations.
TGr MUTATIONS:
5x10e5 cells were seeded in 10-cm dishes and cultivated for 7 days after the treatment. Then the medium was removed, the cells were washed once with PBS and trypsinized. Then 10e5 cells were seeded in 6-cm dishes (10 per concentration) and grown in 5 mL complete medium including 10.9 ug/mL of 6-thioguanine (TG). Cell survival tests were carried out in parallel to the selection procedure. Colonies were fixed and stained after 8 days of incubation.

Reliability: (3) invalid
Unsuitable test system (unphysiological, hypertonic test conditions).

12-APR-2006 (145)

5.6 Genetic Toxicity 'in Vivo'

Type: Micronucleus assay
Species: mouse **Sex:** male
Strain: other: ddY
Route of admin.: i.p.

Exposure period: 24 hours after injection
Doses: single injection: 0; 62.5; 125; 250; 500 mg/kg; four injections within 24 h: 31.3; 62.5; 125; 250 mg/kg
Result: negative

Method: other
GLP: no data
Test substance: other TS: Ammonium chloride, purity 99.7%

Remark: There are no in vivo data on genotoxicity for ammonium sulfate. To bridge the data gap, data for ammonium chloride, which dissociates in aqueous media to form ammonium ions, as does ammonium sulfate, will be used. However, data on sulfate ions are not available.

Result: MNPCE: single injection: 0.12 %; 4 injections: 0.17 %
Test condition: - The maximum dose of ammonium chloride was determined by pilot experiments using the multisampling at multi-dose levels method.Dose up to MTD (maximum tolerated dose) were used.
-Mice were killed 24hr after an administration.
-Femural marrow cells were flushed out with fetal bovine serum and fixed with methanol and stained with Giemsa.
-One thousand polychromatic erythrocytes per mouse were scored using a light microscope and the number of micronucleated erythrocytes (MNPCE) was recorded.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
10-APR-2006 (146)

5.7 Carcinogenicity

Species: rat **Sex:** male
Strain: Fischer 344/DuCrj
Route of administration: gavage
Exposure period: 16 hours
Frequency of treatment: single treatment
Doses: 0, 500, 1000, 1500 or 2660 mg ammonium sulfate/kg bw (7.56-20.1 mM/kg bw) in a volume of 0.5-1 mL by stomach tube
Control Group: yes, concurrent no treatment

Method: other: see Test Condition
Year: 1989
GLP: no data
Test substance: other TS: ammonium sulfate, reagent grade (Wako Pure Chemicals, Osaka)

Remark: These data need careful evaluation as high salt concentrations were given as a bolus dose directly into the stomach. It is known that high salt concentrations can denature proteins leading to cell injury or cell death. Subsequently cell proliferation might occur as a repair mechanism causing an increase in ornithine decarboxylase in the glandular stomach.
Result: The effect of various salts including ammonium sulfate on ornithine decarboxylase in rat stomach mucosa is reported.
A 73 fold increase in ornithine decarboxylase activity was

measured with a maximum after 16 hours. In comparison, an equimolar dose of NaCl (25.7 mmol = 1500 mg/kg bw) induced a 248 fold increase in enzyme activity.
The authors concluded that the various tested salts may have the capability of tumor promotion in the glandular stomach of rats.

Test condition: Male Fischer 344 rats, 7 to 8 weeks old, were given a limited amount of diet (4 g per rat) overnight to reduce their stomach contents. The following day they were given 500, 1000, 1500 or 2660 mg ammonium sulfate/kg bw in a volume of 0.5-1 mL by stomach tube.
Ornithine decarboxylase activity (ODC) was measured in duplicate assays on pooled pyloric mucosa material from four rats 8 h after dosage. ODC activity was determined using L-(1-14C)-ornithine as a substrate. DNA synthesis in the pyloric mucosa of the stomach was determined in in vitro culture.

Reliability: (2) valid with restrictions
limited documentation

Flag: Critical study for SIDS endpoint

10-APR-2006 (147)

Species: Syrian hamster **Sex:** male
Route of administration: inhalation
Exposure period: 15 weeks
Frequency of treatment: 6 hours/day, 5 days/week
Post exposure period: 2 years
Doses: 186.6 ug ammonium sulfate/m3 (analytical) [200 ug/m3 (nominal)]
Result: negative
Control Group: other: BaP (5 mg i.tr.), once a week

Method: other: see Test Condition
Year: 1978
GLP: no
Test substance: other TS: ammonium sulfate, not further specified

Remark: The study evaluated the influence of ammonium sulfate as a cofactor in carcinogenesis studies employing benzo(a)pyrene as the prime agent. The study was repeated with the same concentration and animal number. The dose of ammonium sulfate tested was select as a conservative extrapolation of known sulfate levels of major metropolitan areas (3 fold dose). Additional information concerning influence on a metabolic enzyme (aryl hydroxylase) and the body`s absorption and excretion of sulfate was also included (cf IUCLID section on toxicokinetics).
NOTE: wrong units (mg instead of ug) were used in some parts of the publication by Godleski et al., 1984.

Result: Exposure to ammonium sulfate resulted in a significant depression ($p < 0.05$) of benzo(a)pyrene (B(a)P) carcinogenesis in the first 6 months of the study. At termination of the study, however, there were no differences in cancer incidence between groups receiving B(a)P and B(a)P plus ammonium sulfate. In addition, at the concentration studied, inhaled ammonium sulfate alone did not significantly increase the incidence or severity of pneumonitis or pulmonary fibrosis in the hamster. This inhalation did increase the incidence of emphysema (16.1 vs 8.6%) but not the severity.

Inhalation of ammonium sulfate did not have any effect on body weights.
The incidence of respiratory cancer was 1.4% in unexposed controls, 2.9% in hamsters exposed to ammonium sulfate alone, 14.4% in those given only B(a)P injections and 11.8% in those given B(a)P injections and exposed to ammonium sulfate. The increased incidence of cancer with B(a)P was statistically significant ($p < 0.005$). The incidence of all cancer was 5.9% in unexposed controls, 4.0% in hamsters exposed to ammonium sulfate alone, 34.6% in those given only B(a)P injections and 27.9% in those given B(a)P injections and exposed to ammonium sulfate.

The B(a)P alone group had increased mortality in the first months after exposure and an increased incidence in respiratory tract malignancy when compared with the BaP/ammonium sulfate group. Histologically, the malignancies seen in the respiratory tract were poorly differentiated squamous carcinomas and undifferentiated large cell malignancies.

Test condition: The study was designed as co-carcinogenicity experiment.

Approximately 10-weeks old animals were divided into the following groups (80 animals/group).
- Benzo(a)pyrene (BaP) alone,
- ammonium sulfate alone,
- BaP/ammonium sulfate together,
- and a control group.

Hamsters receiving BaP alone were given 5-mg doses by intratracheal injections once a week for 15 weeks.

The ammonium sulfate group was exposed for 6 hours/day, 5 days/week for 15 weeks to an average concentration of 186.6 ug/m3 sulfate (nominal concentration: 200 ug/m3, particle size: 0.3um -0.6 um MMAD).
The concentration was chosen as a conservative extrapolation of environmentally relevant levels.

The BaP/ammonium sulfate group was simultaneously exposed to 197.6 ug/m3 for 6 hours/day, 5 days/week, 15 weeks.

The examination of all animals was performed two years later.

The study was repeated with the same protocol.
At necropsy gross observations were made on all internal organs, including the brain, nasal structures and larynx. Histologic sections of the respiratory tract (nose, larynx, bronchi, all lobes of the lung) were prepared and hematoxylin and eosin stained slides were evaluated.

Conclusion: The study showed that ammonium sulfate inhalation had no effect on the development of pulmonary cancer and no effect on the development of other significant pulmonary diseases in hamsters. Ammonium sulfate has not a co-carcinogenic effect.

Reliability: (3) invalid
co-carcinogenicity study, time and concentration insufficient for valid negative carcinogenicity study, only one dose (no

MTD), the interval between termination of exposure and examination was too long.
10-APR-2006 (104) (137)

Species: other: SHE Cells **Sex:**
Result: negative

Method: other: cell transformation test

Remark: no further details available. Secondary citation.
Reliability: (4) not assignable
secondary citation
19-APR-2003 (148)

5.8.1 Toxicity to Fertility

Type: other

Remark: For the endpoint fertility studies with ammonium sulfate were not available.

As Ammonium sulfate dissociated in biological systems studies with other ammonium and sulfate salts can be used to cover these endpoint: A screening study for reproductive/developmental according OECD TG 422 screening study was reported with diammonium phosphate as analogue substance which forms ammonium ions in aqueous solutions. Fully valid fertility studies with analogue compounds containing sulfate ions are however lacking. Two limited studies with sodium sulfate can be used for assessment of fertility.
10-APR-2006

Type: One generation study
Species: mouse
Strain: ICR
Route of administration: drinking water
Exposure Period: from one week prior to breeding until study end (day 21 of second parity)
Frequency of treatment: continuously
Premating Exposure Period
male: no treatment (tap water ad lib)
female: 1 week
Doses: 625, 1250, 2500 or 5000 mg sulfate/L in drinking water
Control Group: yes, concurrent vehicle
NOAEL Parental: = 5000 mg/l

Method: other: see Test Condition
Year: 1988
GLP: no data
Test substance: other TS: sodium sulfate, reagent grade

Result: Control mice, receiving only distilled water, consumed significantly less than mice receiving sulfate treatments, and sodium-control mice drank significantly more water than

mice treated with sulfate. No differences were found in litter size, litter weaning weights, or gestational or lactational weight gain of the dams among sulfate treatments.

Test condition: 60 random-bred ICR female virgin mice were used. Mice were assigned randomly to one of six water treatments (control, Na control, 4 dose levels of sodium sulfate). All groups contained equal levels of Na, which was maintained by varying the amount of sodium bicarbonate added. The treated water was available ad libitum beginning 1 wk prior to breeding and was continued throughout the experiment.
After 1 wk of acclimation to the treatments, a male mouse that had received tap water was paired randomly with each female mouse. The females were checked every 24 hours in the morning for the presence of a vaginal plug. After a vaginal plug was observed, the male mouse was removed and the female was weighed.
Water consumption was measured daily during the 2nd and 3rd wk of gestation, and the 1st and 2nd wk of lactation. At parturition, the dams were weighed and litter size was recorded. The litters then were standardized to eight pups per litter. At 21 days postpartum, the pups were weaned and the litters and dams were weighed individually. The dams were then rebred at first estrus immediately following weaning. This procedure was carried out over two parities. Only animals that whelped during each parity were used in the analysis. Thus, the number of dams per dose group in the first parity was 4-9, in the second parity it was only 4. Fertility indices were not measured.
Statistical analysis: least squares mean analysis of variance (SAS, 1982). Student`s t-test was used to determine the difference in water consumption.

Test substance: The doses 625, 1250, 250 and 5000 mg sulfate/ litre corresponding to:
625 mg sulfate/l:
first parity
gestation: ca. 250-300 mg sulfate/kg bw
lactation: ca. 450-710 mg sulfate/kg bw
second parity
gestation: ca. 270-360 mg sulfate/kg bw
lactation: ca. 470-840 mg sulfate/kg bw

1250 mg sulfate/l:
first parity
gestation: ca. 480-570 mg sulfate/kg bw
lactation: ca. 930-1450 mg sulfate/kg bw
second parity
gestation: ca. 595-920 mg sulfate/kg bw
lactation: ca. 1390-2040 mg sulfate/kg bw

2500 mg/kg sulfate/l:
first parity
gestation: ca. 1270-1660 mg sulfate/kg bw
lactation: ca. 2290-3180 mg sulfate/kg bw
second parity
gestation: ca. 1500-1875 mg sulfate/kg bw
lactation: ca. 2560-4320 mg sulfate/kg bw

5000 mg sulfate/l

first parity
gestation: ca. 1790-2190 mg sulfate/kg bw
lactation: ca. 3680-5360 mg sulfate/kg bw
second parity
gestation: ca. 2025-2610 mg sulfate/kg bw
lactation: ca. 3910-6560 mg sulfate/kg bw

Reliability: (2) valid with restrictions
limited documentation, low numbers of animals per dose group, only females were treated, no fertility indices were measured.

Flag: Critical study for SIDS endpoint

14-JUN-2004 (149)

Type: other
Species: rabbit
Sex: female
Strain: no data
Route of administration: oral unspecified
Exposure Period: 5-16 months
Frequency of treatment: on alternate days
Doses: 100-200 mg/kg bw

Method: other: see Test Condition
GLP: no
Test substance: other TS: ammonium sulfate, not further specified

Result: Treatment of virgin female rabbits with ammonium carbonate, chloride, hydrophosphate, sulfate, or hydroxide at 100-200 mg/kg bw, 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. There was also enlargement of the uterus, hypertrophy of the breast, and secretion of milk. (no information as to the incidence and magnitude of the effect reported).

Reliability: (4) not assignable
Secondary citation. No further details available (it was not possible to retrieve the original paper).

10-APR-2006 (150)

Type: other: reproductive/developmental toxicity screening test
Species: rat
Sex: male/female
Strain: other: CD
Route of administration: gavage
Exposure Period: see Test Condition
Frequency of treatment: see Test Condition
Premating Exposure Period
male: 2 weeks
female: 2 weeks
Duration of test: see Test Condition
Doses: 0, 250, 750, 1500 mg/kg bw/day
Control Group: yes, concurrent vehicle
other: NOAEL, reproductive toxicity :
= 1500 mg/kg bw
other: NOAEL, developmental toxicity :
= 1500 mg/kg bw

Method: OECD Guide-line 422
Year: 2002
GLP: yes
Test substance: other TS: diammonium phosphate, not further specified

Result: Treatment at all dosages was well tolerated and there were no treatment-related deaths.
A dosage dependent increase in transient post-dosing salivation was apparent, which was considered to be due to the palatability of the test formulations rather than toxicity. A dosage-dependent increase in the number of animals with reddening of the extremities was also apparent mainly during the early stages of treatment.
Body weight gain and food consumption of males at 1500 mg/kg bw/day appeared to be suppressed when compared with the control group, such that gain between weeks 0-5 for this group was 78% of controls. The body weight gain for reproductive subgroup females receiving 1500 mg/kg bw/day was reduced during the first week of gestation, after which the values returned to levels comparable with the control.
Some treatment-related effects on hematology were evident (reduction in activated partial thromboplastin time for males at 750 and 1500 mg/kg bw/day, a non dosage-dependent elevation of alkaline phosphatase levels at 750 and 1500 mg/kg bw/day, reduced glucose and phosphorous levels at 1500 mg/kg bw/day, a dosage-dependent reduction in total protein at 750 and 1500 mg/kg bw/day with a slight elevated albumin/globulin ratio at the top dosage. Changes in females were limited to a decrease in phosphorous levels and a non-significant increase in alkaline phosphatase level at 1500 mg/kg bw). Relative kidney and liver weights for females at 1500 mg/kg bw/day were greater than in the control group, but there were no histological changes associated.
A number of treated animals at 750 and 1500 mg/kg bw/day exhibited horizontal banding on the incisors at necropsy; histological processing of these tissues failed to detect any change in the areas examined suggesting that the banding was restricted to the enamel of the teeth. The only histological findings related to treatment were the inflammatory/degenerative stomach changes in all treated groups that were considered likely to have arisen due to an irritant effect of the test formulations.
There were no changes apparent at behavioral testing.
Mating performance and fertility were unaffected by treatment, and parental treatment had no apparent effect on the offspring to day 4 of age.
Summary of effects on reproduction/development (control, low, mid, high dose):
Females achieving pregnancy: n= 9, 10, 10, 10.
Dams with live young born: n = 9, 10, 10, 10.
Implants/dam (mean): 15.7, 15.7, 14.1, 15.4.
Live pups/dam at birth (mean): 14.8, 14.6, 12.7, 14.0.
Live pups/dam at day 4 (mean): 14.6, 14.3, 12.7, 14.0.
sex ratio (% m) at birth (mean): 54.2, 52.7, 50.0, 48.5.
sex ratio (%m) at day 4 (mean): 54.2, 53.0, 50.0, 48.5.
Male pup weight at birth (mean): 6.4, 6.3, 6.6, 6.3.
Male pup weight at day 4 (mean): 8.7, 8.5, 9.2, 8.7.
Female pup weight at birth (mean): 5.9, 6.0, 6.1, 6.0.

Female pup weight at day 4 (mean): 8.2, 7.9, 8.6, 8.4.
Post-Implantation survival index: 95.2, 93.5, 90.0, 94.8.
Live birth index: 99.3, 99.4, 100.0, 95.9.
Viability index: 98.6, 98.2, 100.0, 100.0.
There were no effects on the time to achieve conception, and pregnancy length.
Necropsy findings in pups were unremarkable.

Test condition: Animals on the study were distributed to two subgroups (toxicity and reproductive). The toxicity subgroup consisted of 5 males and 5 females per group, and these animals received 5 weeks of continuous daily treatment with functional observations and bleeds for hematology and blood chemistry conducted during week 5 of treatment. Organ weights were recorded at termination during week 6 and tissues preserved and processed for microscopic examination. The reproductive subgroup consisted of 10 females and 5 males per group and animals were given continuous daily treatment until termination. When all the animals had received two weeks of treatment, the reproductive subgroup females were paired with the 5 toxicity and the 5 reproductive subgroups males on a one-to-one basis for assessment of fertility and reproductive function. The toxicity subgroup males were allowed up to 14 days cohabitation after which they returned to the toxicity phase of the study. The females were allowed to litter and rear their young until day 4 of age when parent females and litters were subjected to macroscopic necropsy.
All control groups received the vehicle (water purified by reverse osmosis) alone on the same occasions as the treated animals.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
12-APR-2006 (151)

Type: other: sub-chronic
Species: rat
Sex: male/female
Strain: Fischer 344
Route of administration: oral feed
Exposure Period: 13 weeks
Frequency of treatment: continuously
Doses: 0, 0.38, 0.75, 1.5, 3% in diet (corresponding to 0, 222, 441, 886, 1792 mg/kg bw/day in males and to 0, 239, 484, 961, 1975 mg/kg bw/day in females).

GLP: no data
Test substance: other TS: ammonium sulfate, purity not stated

Result: Organ weights: no significant changes in absolute and relative testis weights
Histopathological examinations: no significant pathological changes were found

Test condition: TEST ANIMALS/HOUSING: 5 week old SPF F344/DuCri rats from Charles River Nippon were used and acclimatized for 1 week prior to the start of the experiments (24 +/- 1 degree C, relative humidity 55 +/-5%, 18 air exchanges/day, 12 hour light/dark cycle). Animals were maintained on CRF-1 powder diet. Rats were randomly divided into five groups, each group consisting of 10 males and 10 females.

EXPOSURE TO TEST SUBSTANCE:
The animals were fed CRF-1 powder diet containing concentrations of 0, 0.38, 0.75, 1.5, and 3.0% of ammonium sulfate. The dose levels were set on the basis of results from a previous 2-week study with a dose level of 5% (no further details reported).
ORGANS WEIGHED:
At necropsy, the following organs were weighed and absolute and relative organ weights determined:
males: brain,lung,heart,spleen,liver,adrenal,kidney,testis.
females: brain,lung,heart,spleen,liver,adrenal,kidney.
ORGANS EXAMINED HISTOPATHOLOGICALLY:
males: brain, lung, heart, spleen, liver, adrenal, kidney, testis.

Reliability: (2) valid with restrictions
ovaries not weighed and examined
Flag: Critical study for SIDS endpoint
10-APR-2006 (134)

5.8.2 Developmental Toxicity/Teratogenicity

Species: other **Sex:**

Remark: For the endpoint developmental toxicity studies with ammonium sulfate were not available.

As Ammonium sulfate dissociated in biological systems studies with other ammonium and sulfate salts can be used to cover these endpoints: A screening study for reproductive/developmental according OECD TG 422 screening study was reported with diammonium phosphate as analogue substance which forms ammonium ions in aqueous solutions. Fully valid developmental studies with analogue compounds containing sulfate ions are however lacking. Two limited studies with sodium sulfate can be used for assessment of fertility and developmental toxicity, however, in non of these studies have the fetuses been examined histologically.
10-APR-2006

Species: mouse **Sex:** female
Strain: ICR
Route of administration: gavage
Exposure period: gestation day 8 through 12
Frequency of treatment: daily
Doses: 2800 mg/kg bw/day
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: < 2800 mg/kg bw
NOAEL Teratogenicity: = 2800 mg/kg bw

Method: other: see Test Condition
GLP: no data
Test substance: other TS: sodium sulfate

Result: No evidence of maternal toxicity (mortality, body weight gain), or increased resorption rate was found. The chemical had no influence on pup survival, and no adverse developmental

effects were observed. When compared to controls, birth weight was significantly increased.
All of the confirmed teratogens, also tested in the study did decrease the live-born litter size.

Test condition: Aqueous sodium sulfate was given by gavage to 28 pregnant ICR mice at a dose of 2800 mg/kg bw/day from gestation days 8 through 12.
A single dose level, at or near the level producing overt maternal toxicity in preliminary range-finding studies with non-pregnant animals was administered. Females were allowed to deliver; litter size and weight on the day of birth and 2 days postpartum were recorded, and stillborns were examined macroscopically.

Reliability: (2) valid with restrictions
limited documentation, short exposure period, fetuses were not examined histopathologically

Flag: Critical study for SIDS endpoint

14-JUN-2004 (152)

Species: hen **Sex:**
Strain: Leghorn
Route of administration: other: injection into the air cell of the egg
Exposure period: single injection at the start of incubation
Doses: 10 mg/egg as aqueous ammonium sulfate (maximum 100 uL/egg)
Control Group: no data specified
Result: No adverse effects were reported for ammonium sulfate

Method: other: see Test Condition
Year: 1980
GLP: no data
Test substance: other TS: ammonium sulfate, aqueous solution

Test condition: In a screening teratogenicity study up to 10 mg/egg of aqueous ammonium sulfate was injected (maximum 100 uL/egg) into the air cells of the eggs at the start of preincubation. The LD50 value was determined to be 4.63 mg/egg. Non viable embryos and hatched chicks were examined for gross abnormalities and signs of toxicosis (such as edema and hemorrhage). In a limited number of animals examination of the viscera was performed and animals with malformations that could not be readily classified were further assessed by X-ray examination or Alizarin Red S staining.

Reliability: (3) invalid
no approved test system

08-DEC-2003 (153)

Species: pig **Sex:**
Exposure period: from pre-breeding day 30 through lactation day 28
Doses: 1790, or 3298 mg/L sodium sulfate
Control Group: yes, concurrent vehicle

Method: other: see Test Condition
GLP: no data
Test substance: other TS: sodium sulfate, analytical grade

Result: Sulfate content of water consumed during gestation had no

significant effect on gestation gain, number of pigs per litter at birth (total and live) or average pig and litter birth weights. Lactation gain, number of pigs at 28 days and average pig and litter weights at 28 days were not significantly affected by sulfates in water during lactation. Slightly less saline water was consumed during gestation. However, in lactation, water consumption increased ($p > 0.05$) as total dissolved solids increased. The general condition and performance of the pigs during the 28-day nursing period were similar among groups. No significant differences occurred at 28 days in average daily gain or feed to gain ratio among weaned pigs that received the control water and those that consumed sulfate added water.

Test condition: 31 sows and 27 gilts were divided into three groups that received either tap water (320 mg sulfate/L) or water with sodium sulfate added at 1790 or 3298 mg/L from pre-breeding day 30 through lactation day 28. Sulfate concentration was determined weekly.

Reliability: (3) invalid
limited documentation, no approved test system

11-JUN-2004 (154)

Remark: No effects to the offsprings were observed in the OECD TG 422 screening study with diammonium phosphate. More information see 5.8.1.

05-FEB-2004

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

Endpoint: other: Influence on enzyme activities

Result: The addition of 125-1000 mM ammonium sulfate to rat hepatic washed microsomal preparations markedly stimulated the rate of in vitro metabolism of the hepatocarcinogen dimethylnitrosamine. Ammonium sulfate can reversibly modify conformation of microsomal membranes in such manner as to increase or decrease microsomal enzyme activities.

Reliability: (1) valid without restriction

18-NOV-2003 (155)

Endpoint: other: metabolism, influence on enzyme activities
Species: rat

Result: Intraperitoneal injections of sublethal doses of ammonium sulfate had no effect on five enzymes of the urea cycle nor on the activity of aspartate transcarbamylase. Significant reductions in body weights were seen in most ammonium

sulfate treated groups.
Test condition: Groups of 6 Wistar rats each were treated by intraperitoneal injections as follows:
I - 2 mM ammonium sulfate / kg / day for seven days.
II - 3 mM ammonium sulfate / kg, twice daily, for 10 days.
III - 3 mM ammonium sulfate / kg, 4 daily injections, for 10 days.
IV - 6 mM ammonium sulfate / kg / day for 10 days.
V - 6 mM ammonium sulfate / kg, twice daily, for 10 days.
VI - arginine 30 minutes before 10.6 mM ammonium sulfate / kg for 10 days.
In each group, controls were treated with NaCl injections instead of ammonium sulfate.
In a separate experiment with 6 Wistar rats, normal enzyme activities and urea excretion were measured (carbamyl phosphate synthetase, carbamyl phosphate transferase, argininosuccinate synthetase, argininosuccinase, arginase).
Test substance: ammonium sulfate
Reliability: (2) valid with restrictions
14-JUN-2004 (156)

Endpoint: other: enzyme induction
Species: Syrian hamster
Route of administration: inhalation
No. of animals: 6
Frequency of treatment: 6h/day, 5 days/week and 1, 3, and 10 weeks
Doses: 0.189 mg/m3 (1 and 3 weeks) and 1.35 mg/m3 (1-10 weeks)
Control Group: other: benzo(a)pyrene (5 mg/week, i.tr.) as positive control

Result: Ammonium sulfate inhalation had no effect on lung aryl hydrocarbon hydroxylase activity. Pulmonary macrophage number was not affected by ammonium sulfate inhalation.
Significant induction of the enzyme was found with Benzo(a)pyrene at all analysis periods.
Test condition: This study was designed to investigate levels of aryl hydrocarbon hydroxylase in hamster lungs. Enzyme activity was studied after 1,3 and 10 weeks of exposure.
6 animals per group per time period were studied.
Reliability: (2) valid with restrictions
14-JUN-2004 (104)

Endpoint: other: pulmonary macrophage study
Species: Syrian hamster
Route of administration: inhalation
No. of animals: 6
Exposure Period: 12 hour(s)
Frequency of treatment: once
Doses: 860 ug/m3
Control Group: yes
Observation Period: immediately, 6, 24 hours later

Result: There was no effect on pulmonary macrophages (number, shape) in hamsters exposed to 0.86 mg/m3 for 12 hours and a particle size of 0.3 um.

Test condition: This study was designed to investigate pulmonary defense mechanism. Pulmonary macrophage numbers were evaluated. Six sulfate-exposed hamsters were killed immediately after exposure and 6 and 24 hours later. Control animals were studied in the same manner.
Reliability: (2) valid with restrictions
11-JUN-2004 (104)

Endpoint: other: LDL0
Species: cattle

Test substance: other TS: ammonium sulfate

Result: Experimental results show that a heifer and a cow were killed by giving them 40 g and 150 g of ammonium sulfate respectively, and further showed that the toxic effect after ingestion of the salt was less than after its subcutaneous injection. Signs of poisoning were severe colic, groaning, staggering, forced rapid breathing, a very marked jugularpulse, and death after violent struggling and bellowing. On post mortem examination in cattle, large hemorrhagic patches on mucous membranes of stomach and intestines with edema and ulceration of intestinal mucous membrane. Liver was enlarged, pale and friable; blood fluid lighter in color than normal; and there were numerous petechiae in skin and throughout carcass.
Reliability: (4) not assignable
secondary citation
10-APR-2006 (157)

Endpoint: other: NOEL
Species: cattle
Doses: 1785 mg/kg

Test substance: other TS: ammonium sulfate

Result: Ammonium sulfate did not induce symptoms of any kind in a112 kg calf when given up to 200 g of pure ammonium sulfate as a drench. However, deaths in cattle have been described following contamination of oil-cake with ammonium sulfate. Another case involved deaths of 5 dairy cows which had consumed ammonium sulfate owing to manure drills having been cleaned out on part of pasture.
Reliability: (4) not assignable
secondary literature
08-DEC-2003 (157)

Endpoint: other: LDL0
Species: goat
Doses: 3500 mg/kg bw

Test substance: other TS: ammonium sulfate

Reliability: (4) not assignable
secondary literature
08-DEC-2003 (158)

Endpoint: other: polioencephalomalacia

Species: other: cattle and sheep
Route of administration: oral

Remark: In the latter part of 1991 an unusual neurological disease was recognised on several farms in England. This report describes the case histories and clinical, biochemical and pathological findings in six calves and two lambs aged from two to 44 weeks obtained from five of these farms. Laminar cerebrocortical necrosis and severe bilateral necrosis of the thalamus and/or striatum progressing to cavitation were recognised in the brains. These changes are similar to those of experimental sulphate toxicity. Morbidity rates of 16 to 48% and mortality rates of 0 to 8 % were recorded. The affected animals did not respond to vitamin B1 treatment; the erythrocyte transketolase levels of in-contact cattle and one untreated calf and one untreated lamb were within the normal range. All five farms had recently introduced a proprietary concentrate ration containing ammonium sulfate rather than the normal urinary acidifier ammonium bicarbonate. After this ration was withdrawn no new cases of nervous clinical disease were observed. It is suggested that, in at least some cases, the morphology and topography of lesions may distinguish sulfate induced polioencephalomalacia from that of sporadic thiamine-dependent cerebrocortical necrosis.

Reliability: (3) invalid
no common test system
10-APR-2006 (159) (160)

Endpoint: other: effect of ammonium sulfate on absorption
Species: sheep

Remark: The experiment was conducted to study the relative effects of sudden doses of urea and ammonium sulfate. The study evaluates urea and ammonium sulfate as separate and combined sources of nonprotein nitrogen in mature ewes and feedlot lambs.
Result: Conclusion:
The data indicate that ammonia absorption is less rapid when ammonium sulfate is added to the rumen than when an isonitrogenous quantity of urea is administered.
In this experiment, 9.3 g of urea nitrogen per 100 lb of bw when given as a drench resulted in two of three ewes dying of ammonia toxicity compared with no toxic symptoms when identical doses of nitrogen were given as ammonium sulfate.
The effect of administering urea and ammonium sulfate in combination was similar to a mathematical average of the effects observed when urea and sodium sulfate were administered alone.
Reliability: (3) invalid
no common test system
10-APR-2006 (161)

Endpoint: Neurotoxicity
Species: rat
Strain: other: Lewis **Sex:**
Route of administration: intrafascicular

No. of animals: 216
Doses: 18 %
Result: negative

Result: Ammonium sulfate did not cause irreversible histologic or functional damage in the rat nerve model and was at least as safe as currently approved regional anesthetics in all age groups. Because of its longer action, the use of ammonium sulfate as an anesthetic in pediatric and adult populations warrants further investigation. Additionally, neonatal injection injury is more focal and recovers faster than adults, supporting the clinical observation that neonatal nerve is superior.

Test condition: 216 Lewis rats aged 4 days, 3weeks, or 3 month were anesthetized for exposure of the right sciatic nerve. An intrafascicular (if) injection was delivered into the posterior tibial nerve fascile proximal to the trifurcation using ammonium sulfate and other experimental agents (n=18 rats/age/agent): 10% ammonium sulfate, 0.9% saline (negative control), 5% phenol (positive control).
The volume of injected material was proportional to nerve size: 4-day, 3-week, and adult rats received 0.1, 0.2, or 0.3 mL, respectively. Sham animals underwent sciatic nerve exposure without injection. All animals were followed for 8 weeks with serial walking track analyses to assess functional recovery.
At 2, 4, and 8 weeks postoperatively, one third of the animals (n=6) from each experimental group were sacrificed and their posterior tibial nerves harvested for light microscopy to assess the extent of histologic damage.

Reliability: (2) valid with restrictions
12-APR-2006 (162)

Endpoint: other: ammonium salt poisoning
Species: sheep

Remark: Three groups of sheep were intraruminally injected with ammonium chloride, ammonium sulfate, or a mixture of ammonium chloride, carbonate, phosphate, and sulfate, respectively.
Ammonium sulfate treatment: 8 ewes were given single or multiple doses of 1.0 to 3.5 g/kg bw, 4 ewes were used as negative controls.

Result: The pathologic alterations were similar in all groups of sheep.
General passive hyperemia and numerous petechial and ecchymotic hemorrhages in the musculature, heart, thymus, and lungs were constant gross alterations. The lungs especially were distended and severely congested.
On microscopic examination, the pulmonary lesions included severe hyperemia, hemorrhage, alveolar edema, and alveolar emphysema. In the thymus, there were degeneration and necrosis of Halls corpuscles and centrilobular hemorrhages. Lesions in kidneys included severe generalized cloudy swelling and multiple foci of early coagulative necrosis of the proximal convoluted tubes, general hyperemia of glomerula trufts, and degeneration of the glomerular truft cells.

Reliability: (2) valid with restrictions

10-APR-2006 (163)

Endpoint: other: synergistic effects of ozone or nitrogen and ammonium sulfate
Species: rat
Strain: Sprague-Dawley **Sex:** male

Remark: A hitherto unexpected synergism between the oxidant air pollutants ozone or nitrogen dioxide and respirable-size aerosol of ammonium sulfate was observed during controlled exposure of rats to these substances. in this experiment were examine biochemical and morphometric changes in lungs of rats exposed for 3,7 or 14 days to ozone (0.64-0.96 ppm) with and without of ammonium sulfate aerosol (diameter of ca. 1 um, 5 mg/m3).
After 3 days of mixture exposure, rat lung macrophage precursors (monocytes) increased 2-3 fold, fibroblasts were increased 2 fold, and apparent collagen synthesis rats were increased 2.5 fold, as compared with values from animals exposed to ozone alone. Continued exposure to ozone alone for 7 or 14 days seemed to mimic changes seen 3 days with the mixture of pollutants Total number of lesions per lung was the same for ozone exposure with and without accompanying aerosol; lesions were larger in lungs of rats exposed to ozone plus ammonium sulfate. The authors suggest that the lifetime of free radicals arising from interaction of oxidants such as ozone or nitrogen dioxide with normal molecules within the lung is increased by either local pH changes in the local sulfate concentration caused by inhalation of respirable aerosol.
Ammonium sulfate alone did not increase the collagen synthesis in lung minces of exposed rats, however, exposure time was not given.
Reliability: (1) valid without restriction
application mixtures from ammonium sulfate, ozone and nitrogen oxide, exposure time not given

12-APR-2006 (164) (165) (166) (167)

Endpoint: other: screening in vitro of pulmonary toxins

Remark: A short-term (20h) culture of functionally competent (nitrotetrazolium reductase positive) Clara cells was developed. In this culture the Clara cells were allowed to attach to an extracellular matrix in 96 multiwell plates . Ammonium sulfate were examined for the ability to reduce the attachment efficiency of functionally competent Clara cells and TD50 values were calculated. Ammonium sulfate had no effect to attachment.
Reliability: (3) invalid
no detailed information of results

14-JUN-2004 (168)

Endpoint: other: Clearance system: role of exposure concentration, time and relative acidity
Species: rabbit

Strain: New Zealand white **Sex:**

Result: The response of respiratory region clearance depend on total exposure, i.e., some combination form of c x t and was also related to relative acidity when H2SO4, NH4HSO4 and ammonium sulfate were investigated. Ammonium sulfate exposure (2 mg/m2) to 5 rabbits for 2h per day for 14 days resulted in no statistically significant change in clearance. Respiratory region clearance was measured by evaluating the retention of 85 Sr-tagged polystyrene latex microspheres (3.5 um MMAD) by daily profile scanning.

Reliability: (2) valid with restrictions
No standard study, but well documented

Flag: Critical study for SIDS endpoint

16-JUL-2004 (169)

Endpoint: Cytotoxicity

Remark: Cytotoxicity of ammonium sulfate in aqueous medium was tested in different mammalian cell lines (HeLa, mouse lymphoma (ML) and Buffalo green monkey (BGM) cell lines). The percentage of survival at nominal concentrations of 1 ug NH3-N/mL was 76, 65 and 66% for HeLa, BGM and ML, respectively. The survival rate at 0.5 ug NH3-N/mL was 84, 79 and 84%. Buffalo green monkey cell line (derived from a non tumor tissue) was more sensitive than the tumor derived cell lines. The authors concluded that ammonium sulfate was not severely toxic to the cell lines tested.

Reliability: (4) not assignable
insufficient information to allow assessment of reliability

10-APR-2006 (170)

Endpoint: other: the effect on clearance from respiratory region or lung

Result: In rats, the clearance of radioactively labeled insoluble particles was not influenced by exposure to ammonium sulfate (3.6 mg/m3; MMAD 0.4 um) at low (30-40%) or high (>80%) relative humidities for 4 hours. Exposure to both H2SO4 (MMAD 1.0 um) and Fe2(SO4)3 (MMAD 0.4 um) significantly retarded clearance during a period of 2 to 17 days after exposure at low, but not high relative humidity.

Reliability: (2) valid with restrictions
no standard study

14-JUN-2004 (116)

Endpoint: other: subchronic exposure to a mixture of O3, S02, and Ammonium sulfate on host defenses of mice

Species: mouse

Strain: CD-1 **Sex:**

Remark: Mice exposed 5h/d, 5d/wk up to 103 d, to 0.2 mg ozone/m3, or to a mixture of ozone, 13.2 mg SO2/m3, and 1.04 mg Ammonium sulfate aerosol/m3 showed no significantly greater

susceptibility to group streptococcal aerosol infection relative to filtered air controls. Pulmonary bactericidal activity by alveolar macrophages was significantly enhanced in the lungs of mice exposed to the mixture relative to those inhaling filtered air or O3 alone.
The mixture caused alterations in several host defense mechanism.

Reliability: (3) invalid
mixture exposure, exposure to streptococcal aerosol infection,
10-APR-2006 (171)

Endpoint: other: inhalation mix-exposure
Species: rat
Strain: Sprague-Dawley **Sex:** male/female
Route of administration: inhalation
Exposure Period: 56 day(s)
Frequency of treatment: 4h/day and 4days/week

Reliability: (3) invalid
mix-exposure: Ammonium sulfate, ammonium nitrate and road dust.
12-APR-2006 (133) (172)

Endpoint: other: cardiopulmonary function
Species: sheep
Doses: 1,1 mg/m3

Result: In conscious sheep, tracheal mucous velocity was altered by 20 min exposure to submicron aerosols (1,1 mg/m3) of the ammonium sulfate. Tracheal mucous velocity was measured by following the motion of 20-30 radiopaque Teflon disks deposited within the trachea.
Reliability: (2) valid with restrictions
unusual animal model, data well documented
Flag: Critical study for SIDS endpoint
10-APR-2006 (119)

5.10 Exposure Experience

Result: The acute exposure of asthmatic volunteers to 1000 ug/m3 ammonium sulfate (ACS certified grade) did no significantly decrease specific airway conductance and forced expiratory volume in one second. Flow rates were not altered.
Test condition: 10 asthmatic subjects to 1,000 ug/m3 ammonium sulfate for 16 minutes (aerodynamic diameter: 0.6 to 1 um). NaCl aerosol of similar characteristics, administered by double-blind randomization, served as control.
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
06-APR-2006 (173)

Type of experience: other: controlled study

Result: Exposure to ammonium sulfate showed no significant effects on any pulmonary function measurements.

Test condition: 13 male healthy volunteers were exposed in chambers of the U.S. EPA Human Studies facility to ammonium sulfate (0.133 mg/m3). The median mass diameter for the chemical specimen was 0.55 µm. 10 men were exposed for 4 hours including 15 min treadmill exercise at 30 degrees C and 60 % relative humidity. 19 parameters of pulmonary function (forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV1.0), FEV1.0/FVC, forced expiratory flow at 25 % of FVC (FEF25), forced expiratory flow at 50 % of FVC (FEF50), peak expiratory flow (PEF), forced expiratory flow between 25 % and 75 % of FVC (FEF 25-75), vital capacity (VC), inspiratory capacity (IC), expiratory reserve volume (ERV), forced inspiratory capacity (FIVC), forced air inspired at 1 sec (FIV 1.0), FIV1.0/FIVC, peak inspiratory flow (PIF), forced inspiratory flow between 25 % and 75 % of FIVC (FIF25-75), and forced inspiratory flow between inspired volumes of 200 to 1200 ml (FIF200-1200), functional residual capacity (FRC), airway resistance (RAW), specific airway resistance (SRAW)) were measured just prior to exposure (air control), 2 hr into exposure, following the first exercise session, 4 hr into exposure, following the second exercise session and 24 hr after exposure.

Reliability: (2) valid with restrictions
valid with restrictions; no information on purity

Flag: Critical study for SIDS endpoint

10-NOV-2003 (174)

Type of experience: other: controlled study

Remark: Five normal subjects, 5 sensitive subjects and 6 asthmatics were studied.
Neither significant functional changes nor consistent changes in symptom score were found in the normal group at low humidity. at high humidity, significant variation in forced expiratory measures was detected, but the changes were small in magnitude, and the performance tended to be better on exposure days than on control days.
The sensitive group displayed small but significant variations in pulmonary function at both low and high humidity; once again, these appeared to be occurrences not related to the aerosols. On the last exposure day of the high-humidity studies, the sensitive subjects reported a significant increase in total symptom score. Examination of the individual symptoms reports indicated that his was due to increased fatigue.
The asthmatic subjects showed a marginal decrease in the slope of the alveolar plateau of the single breath N2 washout between the beginning and the end of exposure, both with clean air and with exposure to ammonium sulfate. In the
absence of other changes in function, this would be interpreted as representing an increase in uniformity of ventilation distribution within the bungs, i.e., an improvement in function. Symptom scores and other pulmonary function results did not indicate any increase toward dysfunction.
In conclusion, no evidence of an adverse health effect on healthy, sensitive and asthmatic adult men was found after 2

hour multiple day exposures to "worst case" ambient concentrations (0.1-0.3 mg/m3 of ammonium sulfate with a MMAD of 0.3-0.6 µm and a relative humidity ranging between 40 and 85% in the different experiments).

Test condition: Both healthy, "sensitive" and asthmatic adult men were exposed to nominal (100 µg/m3) concentrations of ammonium sulfate, ammonium bisulfate, and sulfuric acid with a mass median aerodynamic diameter (MMAD) of 0.3 µm and a geometric standard deviation of approximately 3. "Sensitive" subjects had been studied previously and had shown unusually definite reactions in controlled exposures to ozone. Exposure concentrations and particle size distributions of the sulfate salts were based on the highest reported 2-h filter sample of particulate sulfate in the Los Angeles Basin. Groups of 6 subjects were typically exposed, 3 at a time. Exposures were performed in a blind experimental fashion, i.e. subjects were unaware of their actual aerosol exposure day, with 1 or 2 days of a "control" exposure to purified air followed by 2 to 3 consecutive days of sulfate aerosol exposure.
To initiate the 2-h protocol, subjects performed baseline pulmonary function tests on entering the chamber. Subjects then exercised for the first 15 minutes of each 0.5 h for 2 h, on bicycle ergometers to double, approximately, the subject´s minute volume of ventilation (comparable to light outdoor activity). Chamber temperature and relative humidity were maintained at 31 deg C and 40 per cent relative humidity which simulated a typical Los Angeles summer day. Additional exposures to ammonium sulfate were performed at 85 per cent relative humidity. Lung function tests were performed before and after exposures and included FVC, FEV 1.0, maximal mid expiratory flow rate, total lung capacity, residual volume, single-breath nitrogen, and total respiratory resistance. Analysis of subject performance after the exposures to ammonium sulfate revealed no important differences in response between normal and sensitive subjects. The measured ammonium sulfate concentrations were between 0.104 and 0.337 mg/m3, the MMAD ranged from 0.28 to 0.6 µm.

Reliability: (2) valid with restrictions
valid with restrictions; no information on purity

Flag: Critical study for SIDS endpoint

12-APR-2006 (175)

Remark: A cluster of ten cases of Hodgkin`s disease was identified within the active workforce of a large chemical manufacturing firm over a 23-year period. In the total workforce of 62000 person-years, an expected number of 2.01 cases was calculated, giving a standardized incidence ratio of 497 (95 % CI 238-915). A nested case-control study was undertaken with 200 controls. For 11 chemicals (incl. ammonium sulfate) exposure odds ratios were calculated. No substance emerged as a likely candidate for explaining the observed Hodgkin`s disease cluster.

Reliability: (1) valid without restriction

Flag: Critical study for SIDS endpoint

20-MAY-2003 (176)

Type of experience: other: case-report of fatal intoxication

Result: An 85-year-old woman (149 cm, 37.5 kg) was found dead lying on the ground outside of her house in the middle of March. There were no signs of struggle. A small amount of dried vomitus was found around her mouth and on her face and a beer can containing a small amount of a beer-like solution was lying beside her. Examination of the solution from the beer can at the police laboratory showed that it was very likely ammonium sulfate. On the basis of the results from the police laboratory analysis, her house was searched and a bag of fertilizer labeled ammonium sulfate was found in the barn.
External examination of the body showed no injury nor abnormalities. Alcohol was not detected in the blood and no toxicological substances, including pesticides, were detected by a routine poison examination with TOXI-LAB. Heart, lung, liver and kidney did not show any pathological findings on macro- and microscopical examination. The lower part of the esophagus mucosa was brownish and contained a foamy fluid.
There was mild petechial hemorrhage in the fundic mucosa without any erosion or corrosion. the stomach contained mostly fluid with pH of 7.0 and tiny bubbles. The autopsy could not determine the definite cause of death and it was assumed that she froze to death.
In serum, ammonium and sulfate ions were significantly increased (25,000 ug ammonium/dL, 12 mEq sulfate/L; normal values: < 30-80 ug/dL for ammonium, < 0.25-0.35 mEq/L for sulfate). Also, ammonium sulfate was detected in the gastric contents.
It was concluded, that the cause of death was acute intoxication due to ingesting ammonium sulfate dissolved in beer for the purpose of committing suicide.

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
12-APR-2006 (105)

Type of experience: other: controlled study

Result: Pulmonary function (body plethysmography and spirometry) and bronchial reactivity (to metacholine) was not affected in 20 non-smoking subjects after a 4-hour exposure of 528 +/- 39 µg/m3 ammonium sulfate aerosol. The exposure period included two 15-minute light to moderate exercise stints per day in the exposure chamber. The subjects served as their own control and breathed clear air on one day before and one day after the exposure to ammonium sulfate.
Method: environmentally controlled chamber with a ventilation rate 8.49 m3/min enable complete air change every 2.6 min; mass median diameter (MMD) for the sulfate aerosol was 0.97 +/- 0.05 µm. Measure of response: FVC, FEV1, and FEV3 were manually calculated from the spirogram; air way resistance(Raw) and volume of thoracic gas (Vtg) were determined by the whole-body pressure plethysmographic technique of Dubois; methacholine challenge was performed according to the method of Chai.

Reliability: (2) valid with restrictions
limited documentation, no information on purity

Flag: Critical study for SIDS endpoint
14-JUN-2004 (177)

Remark: Total nuisance dust air concentrations were measured during bagging and bulk loading of fertilizer, ammonium sulfate and
ammonium phosphate. Four samples showed nuisance dust concentrations ranging from none to 0.38 mg/m3. At a follow-up survey, six environmental air samples collected ranged from 2.55 to 11.1 mg/m3 (only one sample exceeded 10.0 mg/m3). Recommendations were made to reduce exposure to
nuisance dust and improve dust control.

Reliability: (2) valid with restrictions
limited documentation; no follow-up results (i.e. after implementation of recommended measures) available.
20-MAY-2003 (178)

Remark: Ammonium sulfate elicited significant changes in pulmonary flow resistance (34 % decrease) and dynamic lung compliance (17 % decrease) in human volunteers. These changes appeared late in exposure (120 minutes and 90 minutes, resp.) and were partially reversed after 30 minutes recovery.
Method: 4 healthy adult non-smoking human volunteers were exposed to 1 mg/m3 ammonium sulfate (droplet aerosol, MMD 0.71 µm GSD 1.8, rel. humidity > 80 %) Exposure consisted of a 30-40 min. baseline period of room air followed by two hours of exposure (oral breathing) and a subsequent 45 min. recovery period. Pulmonary function was measured every 30 min.

Reliability: (2) valid with restrictions
valid with restrictions; substance purity not given

Flag: Critical study for SIDS endpoint
10-APR-2006 (122)

Type of experience: other: case-control study

Method: A self-administered questionnaire was used to obtain information on past occupation and smoking status. The following chemical exposures were listed in the questionnaire for a yes/no response: organic solvent, lead, cadmium, nickel, chromium, asbestos, acrylonitrile, arsenic,
and beryllium (NOTE: ammonium sulfate was NOT included). A free format question was also included in the questionnaire for obtaining information on other exposures.
The past history of occupational exposures, which was obtained by yes/no questions from selected substances as well as by the free question was classified into 6 categories: inorganic acid/base, asbestos, dust excluding asbestos, organic chemicals, metals, and others, and relative risks were estimated by a conditional logistic model controlling for smoking and concomitant exposures. The
workers exposed to inorganic acid/base showed a significant increase in risk at the 5% level (RR = 4.03). The chemicals classified into this category were sulfuric acid,

hydrochloric acid, phosphoric acid, ammonia, ammonium sulfate, and lime.

Result: A hospital based case control study was conducted in an industrialized Japanese city to evaluate occupational risk factors for lung cancer. A total of 144 lung cancer cases (117 males, 27 females) and 676 comparisons (479 males, 197 females) were identified from 3 major hospitals. A self administered questionnaire was used to gain information on lifetime occupational histories and smoking status. Seven high risk jobs were selected: mining, steel manufacturing, iron working, building construction, road construction, shipbuilding, and industrial plant jobs. Relative risks (RR)
were estimated statistically and the interaction of occupational risk and smoking was evaluated by additive and multiplicative models. No increase in RR was evident for coal miners and nonsignificant elevations were seen for building and road construction workers, however, significant
elevations were noted for ship builders, chemical factory workers, and ironworkers. According to the study authors, the following chemicals were associated with increased risks: sulfuric acid, hydrochloric acid, phosphoric acid, ammonia, ammonium sulfate and lime. The effects of smoking and iron works employment followed an additive model, while that of smoking and other factory work followed a multiplicative model. The authors conclude that RR is increased for shipbuilders, ironworkers, and chemical factory personnel, but that determination of precise risk factors requires more research.

Reliability: (3) invalid
The publication does not contain any detail with regard to ammonium sulfate exposure other than that this chemical was classified together with strong acids (e.g. sulfuric acid) and bases into the "inorganic acid/base" category. The total
number of exposed in the "inorganic acid/base category" was only five persons (and 5 controls). No causal relationship between ammonium sulfate exposure and an increase in relative
lung cancer risk can be deduced from the presented data.

12-APR-2006 (179)

Remark: Gastrointestinal dysfunction giving symptoms similar to acute dysentery was observed in a group of 18 people who drank water containing 1500 - 2000 mg/L ammonium sulfate. An increased sulfate concentration was also found in the vomit (up to 800 - 1000 mg/l). The water was drunk from a faucet located next to vegetable hothouse where the ammonium sulfate was used as fertilizer. All of those affected were in satisfactory condition after 24 hours.

Reliability: (4) not assignable
documentation insufficient for assessment

25-NOV-2003 (180)

Type of experience: other: controlled study

Result: At 1 mg/m3, none of the inhaled sulfates produced

significant decreases in specific airway conductance (SGaw) in normal subjects but did produce small changes in flow on the maximum expiratory flow-volume (MEFV) and partial expiratory flow-volume (PEFV) curves ($p<0.05$) (no quantitative data are provided for this effect for the single chemicals tested).
Carbachol bronchoconstriction (decreases in specific airway conductance) was enhanced in relation to the acidity of the sulfates.

Test condition: 16 laboratory workers (mean age 27 years) inhaled a control NaCl aerosol and the following sulfates: ammonium sulfate, sodium bisulfate, ammonium bisulfate and sulfuric acid. All subjects were non-smokers. A Lovelace generator produced particles with an average MMAD of approximately 0.5-1.0 µm (GSD 1.5-2.2) and concentrations of 0.1 and 1 mg/m3 (as sulfate). The relative humidity of the aerosol was kept between 20 and 25% to ensure that all aerosols consisted of dry particles. By double-blind randomization all subjects breathed the aerosols for a 16 minute period, with at least 3 hours separation between exposures. To determine if sulfate inhalation caused increased reactivity to a known bronchoconstrictor, all subjects inhaled carbachol following each 16-minute exposure. Before and after each exposure, the subject`s pulmonary function was assessed in a body plethysmograph and a dose-respose curve to inhaled carbachol was constructed for all subjects at the beginning of each study.

Reliability: (2) valid with restrictions
valid with restrictions, no quantitative data provided for effect on airway conductance

Flag: Critical study for SIDS endpoint

12-APR-2006 (181)

Result: Report on skin irritation in a woman after contact with pool
water treated with ammonium sulfate, sodium carbonate, and chlorine. Skin irritation was produced one day after dipping
of one arm or splashing the water onto legs and the other arm.

Reliability: (4) not assignable
documentation insufficient for assessment; mixed exposures

10-APR-2006 (182)

Remark: If ingested in very large quantities, ammonium sulfate may produce ammonium poisoning which causes tremors, slurred speech, loss of balance, stupor and coma.

Reliability: (4) not assignable
only secondary literature, data from a MSDS

14-JUN-2004 (183)

Remark: The response of nine asthmatic subjects who were 60 to 75 years of age to inhaled sulfuric acid and ammonium sulfate was investigated and compared to eight healthy subjects. Each subject was exposed to clean air, 70 µg/m3 ammonium

sulfate aerosol, or 70 µg/m3 sulfuric acid during a 40-min exposure period composed of 30 min at rest and 10 min of light exercise.70 µg/m3 of ammonium sulfate or sulfuric acid. The MMAD of the aerosol was 0.6 µm. Oral ammonia levels and pulmonary function parameters (forced expiratory volume in one second, forced vital capacity, and total respiratory resistance) were measured before and after each exposure. None of the functional parameters in either group showed significant changes. However, total respiratory resistance changes from baseline after sulfuric acid exposure were significantly higher (+16%) in the asthmatic subjects, compared with the healthy subjects (-6%).

Reliability: (2) valid with restrictions
valid with restrictions

Flag: Critical study for SIDS endpoint

10-APR-2006 (184)

5.11 Additional Remarks

Type: adsorption

Reliability: (2) valid with restrictions
reliable review

26-AUG-2005 (11)

Type: adsorption

Reliability: (2) valid with restrictions
reliable review

10-APR-2006 (12)

Type: other: Review

Remark: The health effect of sulfur dioxides and particulate matter in ambient air is reviewed.

Reliability: (4) not assignable

10-APR-2006 (185)

Type: other: occupational exposure

Remark: Exposure of workers to ammonium sulfate may occur during production, transport and processing, or through the professional use of ammonium sulfate containing products. Main exposure routes are the respiratory route (inhalation of aqueous aerosols or dust), or dermal contact with the solid. Exposure of workers to dust during production and storage, loading and unloading of trucks was measured. Eight measurements of respirable dust (including fine dust fraction) were carried out within 8 h (personal air samplers and sampling from the working room), but no analysis for ammonium sulfate performed, assumed as 100 %.
Results: all measurements showed concentrations close to the detection limit. Personal air samplings for respirable dust showed values < 0.354 - 0.360 mg/m3 (four samples) and for fine dust < 0.442 - 0.455 mg/m3 (four samples).

Reliability: (2) valid with restrictions

12-APR-2006 (186)

6.1 Methods Handling and Storing

Common Storage: Segregate from alkalies and alkalizing substances.
Segregate from nitrites and alkaline substances.
Transport Code: Not classified as hazardous under transport regulations.

Remark: PERSONAL PROTECTIVE EQUIPMENT

Respiratory protection:
Breathing protection if breathable aerosols/dust are formed.
Particle filter EN 143 Type P1, low effiency (solid particles of inert substances).

Hand protection:
Chemical resistant protective gloves (EN 374)
e.g. nitrile rubber (0.4 mm), chloroprene rubber (0.5 mm), polyvinylchloride (0.7 mm) and other

Eye protection:
Safety glasses with side-shields (frame goggles) (EN 166)

General safety and hygiene measures:
Handle in accordance with good industrial hygiene and safety practice.
Flag: non confidential, Critical study for SIDS endpoint
18-MAY-2004 (1)

6.2 Fire Guidance

Hazards: harmful vapours
Add. Information: Product itself is non-combustible; fire extinguishing method of surrounding areas must be considered.
Products arising: ammonia

Remark: Thermal decomposition product at > 235°C: ammonia
Flag: non confidential, Critical study for SIDS endpoint
18-MAY-2004 (1)

Products arising: ammonia

Remark: When heating solid ammonium sulphate above 100°C, ammonium hydrogensulphate ((NH4)HSO4) is formed under release of ammonia (NH3).
Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (2)

6.3 Emergency Measures

Type: other: General advice: Remove contaminated clothing

Flag: non confidential, Critical study for SIDS endpoint

26-MAY-2003 (1)

Type: injury to persons (inhalation)

Remark: If difficulties occur after dust has been inhaled, remove to fresh air and seek medical attention.
Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (1)

Type: injury to persons (skin)

Remark: Wash thoroughly with soap and water.
Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (1)

Type: injury to persons (eye)

Remark: Immediately wash affected eyes for at least 15 minutes under running water with eyelids held open, consult an eye specialist.
Flag: non confidential, Critical study for SIDS endpoint
26-MAY-2003 (1)

Type: accidental spillage

Remark: Environmental precautions:
Do not empty into drains, Retain and dispose of contaminated wash water.

Methods for cleaning up or taking up:
For large amounts: Sweep/shovel up.
For residues: Rinse away with water.
Flag: non confidential, Critical study for SIDS endpoint
18-MAY-2004 (1)

6. REFERENCES

(1.)BASF AG (2003). Safety data sheet AMMONIUM SULPHATE 21%N GROB. 30.04.2003 (30041387)

(2) RÖMPP Online (2003). Georg Thieme Verlag, status 20.11.2003

(3) BASF AG (2000). Technical Leaflet AMMONIUM SULFATE GRADES, April 2000

(4) IFIA (2002). International Fertilizer Industry Association,"Ammonium Sulphate Statistics 2002", Paris (France)

(5) Speight, James G. (2002). "Chemical and process design handbook", McGraw-Hill, ©2002

(6) Ullmann (2000). Ullmann's Encyclopedia of Industrial Chemistry, Sixth Edition, 2000 Electronic Release

(7) TRGS 900 (2001). Technical guidance for hazardous substancesTechnische Regeln für Gefahrstoffe (Germany) of 09/2001

(8) Umweltbundesamt (2003). Catalogue of Substances Hazardous to Water - Umweltbundesamt Berlin, status 13.03.2003

(9) National Chemical Inventories (2001). Issue 2

(10) BASF AG (2003), internal communication, 25.03.2003

(11) WHO (1986). Environmental Health Criteria 54. Ammonia. Published under the joint sponsorhip of the United Nations Environment Programme, the International Labour Organisation and the World Health Organization. Geneva, 1986.

(12) WHO (1996). Guidelines for drinking-water quality, 2nd ed. Vol. 2. Health criteria and other supporting information. Geneva

(13) Gmelin (1936). Gmelins Handbuch der anorganischen Chemie pp. 261-282.

(14) CRC (2002). Handbook of Chemistry & Physics, CRC Press. 83rd. edition, 1 Febr 2002

(15) BASF AG (1999). Material Safety Data Sheet Ammonium Sulphate 21 % N grob. 27 December 1999.

(16) BASF AG (2003). Product Safety and Regulations Department. Unpublished calculation. 22 April 2003.

(17) Scott WD and Cattell FCR (1979). Vapor Pressure of Ammonium Sulfates. Atmospheric Environment 13, pp 307-317.

(18) United Nations Industrial Development Organization (UNIDO) and International Fertilizer Development Center (IFDC) (1998). Fertilizer Manual. Kluwer Academic Publishers, The Netherlands.

(19) BASF AG (2002). Product Information. Ammonium Sulfate Industrial Grade.

(20) BASF AG (1988). Department of Analytics. Unpublished study BRU 88.222. 04 November 1988.

(21) BASF AG (2004). Department of ecology, unpublished calculation, 26.01.2004

(22) Lide DR (1992). CRC Handbook of Chemistry and Physcics. Boston. CRC Press.

(23) Frank R (1980). Coal Consumption, Ambient Air Pollution and the Lung. Health Implic New Energy Technol (Park City Environ Health Conf), 347-358.

(24) National Fire Protection Association (1978). In: European Commission (2000). Ammonium Sulphate. International Uniform Chemical Information Database. Year 2000 CD-ROM edition.

(25) BASF AG (1999). Material Safety Data Sheet Ammonium Sulphate 21 % N grob. 27 December 1999.

(26) Gmur NF, Evans LS, Cunningham EA (1983). Effects of ammonium sulphate aerosols on vegetation - II. Mode of entry and responses of vegetation. Atmospheric environment 17(4),715-721.

(27) Holt GJ, Arnold CR (1983). Effects of Ammonia and Nitrite on Growth and Survival of Red Drum Eggs and Larvae. Transactions of the American Fisheries society 112, 314-318.

(28) Rice SD and Bailey JE (1980). Survival, size, and emergence of pink salmon, Oncorhynchus gorbuscha, alevins after short- and long-term exposures to ammonia. Fishery Bulletin. 78, 641-648.

(29) Sinclair JD, Psota-Kelty LA, Peins GA, Ibidunni AO (1992). Indoor/outdoor relationships of airborne ionic substances: comparison of electronic equipment room and factory environments. Atmospheric Environment 26A(5), 871-882.

(30) Leaderer BP, Naeher L, Jankun Th, Balenger K, Holford TR, Toth C, Sullivan J, Wolfson JM, Koutrakis P (1999). Indoor, Outdoor, and regional summer and winter concentrations ofPM10 , PM25, SO42-, H+, NH4+, NO3-, NH3, and nitrous acid in homes with and without kerosene space heaters. Environmental Health Perspectives 107(3), 223-231.

(31) UBA (2003). Environmental Data Germany 2002.

(32) Weijers EP, Vugts HF (1990). The composition of bulk precipitation on a coastal island with agriculture, compared with an urban region. Atmospheric Environment 24A(12), 3021-3031.

(33) EU (1995). European Parliament and Council Directive No 95/2/EC of 20 Feb 1995 on food additives other than sweeters, (OJL61, 18.3., 1995, p1, Annex V), amended by Directive 2003/52/EC of European Parliament and Council of 18 June 2003, p23, 17.7. 2003.

(34) FDA (2003). Code of Federal Regulations: Food and Drugs Part 182 and 184, Office of the Federal Register. National Archives and Records Administration. Washington, DC.

(35) WHO (2003). Guidelines for Drinking Water Quality, 3rd ed Vol.2. Health criteria and other supporting information. Geneva

(36) NAS/NRC Food Additives Surveys (1981). National Academy Press, Washington DC. As cited in HSDB, 04/2001.

(37) Berden M, Nilsson SI (1996). Influence of added ammonium sulphate on the leaching of aluminium, nitrate, and sulphate a laboratory experiment. Water, Air, and Soil Pollution 87, 1-22.

(38) Perrin TS, Boettinger JT, Frost DT, Norton JM (1998). Decreasing Nitrogen leaching from sandy soil with ammonium-loaded clinoptilolite. J Environ Qual 27, 656-663.

(39) Sveda R, Rechcigl JE, Nkedi-Kizza P (1992). Evaluation of various nitrogen sources and rates on nitrogen movement, Pensacola bahiagrass production, and water quality. Commun. Soil Sci. Plant Anal. 23(17-20), 2451-2478.

(40) David M, Vance G, Kahl J (1999). Chemistry of dissolved Organic carbon at bear Brook Watershed, Maine: Stream Water Response to (NH4)2SO4 Additions. Environmental Monitoring and Assessment 55, 149-163.

(41) Nadelhoffer K, Downs M, Fry B, Magill A, Aber J (1999). Controls on N retention and exports in a forested watershed Environmental Monitoring and Assessment 55, 187-210.

(42) Shahandeh H, Cabrera L, Sumner ME (1992). Nitrogen mineralization and nitrification in soils treated with ammonium sulfate and nutrasweet sludge. Commun Soil Sci Plant Anal 23 (15&16), 1923-1934.

(43) Shahandeh H, Cabrera L, Sumner ME (1992). Ammonia Volatilization from Urea, ammonium sulfate, and Nutrasweet sludge in bare and straw covered soils. Commun Soil Sci Plant Anal 23 (7&8), 775-786.

(44) Moal JF, Martinez J, Marol C, Guiraud G (1994). A direct incorporation of N-15 labelled ammonium sulphate into a pig slurry: a laboratory experiment on NH3 volatilization. Bioresource Technology 48, 87-89.

(45) Debreczeni K, Berecz K (1998). Monitoring of gaseous nitrogen losses from nitrogen fertilizers in model experiments. Commun Soil Sci Plant Anal 29(1), 2207-2216.

(46) Carnol M, Ineson P, and Dickinson ML (1997). Soil solution nitrogen and cations influenced by (NH4)2SO4 deposition in a coniferous forest. Environmental Pollution 97(1-2), 1-10.

(47) Carnol M, Ineson P, Anderson JM, Beese F, Berg MP, Bolger T,Coûteaux MM, Cudlin P, Dolan S, Raubuch M, Verhorf HA(1997). The effects of ammonium sulphate deposition and root sinks on soil solution chemistry in coniferous forest soils. Biogeochemistry 38, 255-280.

(48) EPA (1995). U.S. Environmental Protection Agency: Ammonia; Ammonium Sulfate (Solution); Ammonium Nitrate (Solution);Water Dissociable Ammonium Salts; Toxic Chemical Release Reporting; Community Right-to-Know. 40 CFR Part 372, April 03, 1995.

(49) BASF AG (1989). Department of Toxicology, unpublished report (88/736), 27 April 1989.

(50) Larsson A and Linden E.(1978). Results and Experiences of the "Iso Ringtet" on Determination of Acute toxicity to Fish. Nordfolk Miljoevardssekr. Publ. 2, 303-312.

(51) Thurston RV, Russo RC (1983). Acute toxicity of ammonia to rainbow trout. Transactions of the American Fisheries Society 112, 696-704.

(52) Banerjee TK (1993). Estimation of acute toxicity of ammonium sulphate to the fresh water catfish, Heteropneustes Fossilis. I. Analysis of LC50 values determined by various methods. Biomedical and Environmental Sciences 6, 31-36.

(53) IVL (1978). Inst. foer Vattn- och Luftvardsforskning, Stockholm, Sweden, report B 425.

(54) Sörensen L and Landner L (1978). The ISO Ringtest on determination of acute toxicity to fish. Institut för Vatten- och Luftvardsforshkning. February, 1978.

(55) Chouhan MS and Pandey AK (1987). On the tolerance limits and toxicity symptoms of guppy (Lebistes reticulatus) to fertilizers: urea and ammonium sulphate. Comp. Physiol. Ecol. 12(2), 77-80.

(56) Paul VI and Banerjee TK (1996). Ammonium sulphate induced stress related alterations in the respiratory epithelium of the airbreathing organ of the catfish Heteropneustes fossilis (Bloch). J. Biosci. 21 (4), 519-526.

(57) Bengtsson BE and Tarkpea M (1983). The acute aquatic toxicity of some substances carried by ships. Marine Pollution Bulletin 14, 213-214.

(58) Lindén E et al. (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.

(59) Ewell WS et al. (1986). Simultaneous Evaluation of the Acute Effects of Chemicals on Seven Aquatic Species. Environmental Toxicology and Chemistry 5, 831-840.

(60) Franklin F (1980) Assessing the Toxicity of Industrial Wastes, with Particular Reference to Variations in Sensitivity to Test Animals. Fisheries Research Technical Report Number 61. Ministry of Agriculture, Fisheries and Food

(61) Sarkar SK (1991). Toxicity Evaluation of urea and ammonium sulphate to Oreochomis Mossambicus (Peters). J Ecobiol 3 (1), 79-80.

(62) Konar SK and Sarkar SK (1983). Acute toxicity of agricultural fertilizers to fishes. Geobios. 10, 6-9

(63) Paul VI and Banerjee TK (1995). Acute toxicity of ammonium sulphate to the air-breathing organ of the live fish Heteropneustes (Saccobranchus) fossilis (Bloch). Current Science, 68 (8), 845-849.

(64) Paul VI and Banerjee TK (1996). Ammonium sulphate induced stress related alterations in the opercular epidermis of the live fish Heteropneustes (Saccobranchus) fossilis (Bloch).Current Science 70 (11), 1025-1029.

(65) Pramanik A and Sarkar SK (1987). Comparative Study of the Sensitivity of Egg, Spawn and Fry of Cyprinus Carpio Exposed to Ammonnium Sulphate at Different Tempertures. Geobios 14 229-230.

(66) Sheehan RJ and Lewis WH (1986). Influence of pH and ammonia salts on ammonia toxicity and water balance in young channel catfish. Transcriptions of the American Fisheries Society 115, 891-899.

(67) Nebeker AV and Schuytema GS (2000). Effects of Ammonium Sulphate on Growth of Larval Northwestern Salamanders, Red-Legged and Pacific Treefrog Tadpoles, and JuvenileFathead Minnows. Bull Environ. Contam. Toxicol. 64, 271-278.

(68) Svec J (1984). [The influence of Industrial nitrogen Fertilizers on Some Aquatic Animals]. Ceskoslovenska Hygiena 29, 141-147 [Czechoslovakian].

(69) Knoph MB (1992). Acue toxicity of Ammonia to Atlantic Salmon (Salmo salar) parr. Comp. Biochem. Physiol. 101C(2), 275-282.

(70) D'Silva C, Verlencar XN (1976). Relative Toxicity of Two Ammonium Compounds found in the Waste of Fertilizer Plants. Mahasagar 9, 41-44.

(71) Van Vuren JHJ(1986). The effects of toxicants on the haematology of Labeo Umbratus (Teleostei: Cyprinidae). J Comp Biochem Physiol 83C, 155-159.

(72) Sarkar SK (1988). Influence of Ammonium Sulphate on the feeding rate of fish under multivariate temperature. PComp Physiol Ecol 13(1), 30-33.

(73) Reddy NA, Menon NR (1979). Effects of ammonia and ammonium on tolerance and byssogenesis in Perna viridis. Marine Ecology Progress Series 1, 315-321.

(74) Johnson WW and Sanders HO (1977). Chemical forest fire retardants. Acute toxicity to five freshwater fishes and a scud. Technical Papers of the U.S. Fish and Wildlife Service.

(75) Tchounwou PB et al. (1991). Toxicity evaluation of ammonium sulphate and urea to three developmental stages of freshwater snails. Arch. Environ. Contam. Toxicol. 21(3), 359-364.

(76) Chetty AN and Indira K (1995). Free radical toxicity in a freshwater bivalve, Lamellidens marginalis under ambient ammonia stress. Journal of Environmental Biology 16(2), 137-142.

(77) Tchouwou PB, Englande AJ Jr, Malek EA, Anderson AC, Abdelghani AA (1991). The effects of ammonium sulphate and urea upon egg hatching and miracidial survival of Schistosoma mansoni. J. Environ. Sci. Health B26(2), 241-257.

(78) Sarkar SK, Pramanik A (1986). Evaluation of the toxicity of two nitrogenous compounds on freshwater invertebrates exposed to different temperature. J Environ Biology 7(3), 183-188.

(79) Abdel-hamid AZ, Farrag E, Rizk M, Omar MT, Mantawy MME (1997). Effect of nitrogen, sulpher, and phosphorous containing fertilizers and organophosphorous insecticide on reproduction of Biomphalaria alexandria snails. Egypt J Pharm Sci 38(1-3), pp 147-158.

(80) Tam NFY and Wong YS (1996). Effect of ammonia concentrations on growh of Chlorella vulgaris and nitrogen removal from media. Bioresource Technology 57, 45-50.

(81) Thomas WH, Hastings J, and 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. Marine Envrionm. Res. 3, 291-296.

(82) Amat MA and Braud JP (1990). Ammonium uptake by Chondrus crispus Stackhouse (Gigartinales, Rhodophyta) in culture. Hydrobiologia 204/205, 467-471.

(83) Gupta RK (1986). Effect of Different Nitrogen Sources on Growth of the Unicellular Blue-Green alga Synechococcus Cedrorum. Banglades J. bot. 15(2), 159-162.

(84) Fernández Valiente E, Ucha A, Quesada A, Leganés F, Carreres R (2000). Contribution of N2 fixing cyanobacteria to rice production: availability of nitrogen from 15N-labelled cyanobacteria and ammonium sulphate to rice. Plant and Soil 221, 107-112.

(85) Martensson M, Witter E (1990). Influence of various soil amendments on nitrogen-fixing soil microorganisms in a long term field experiment, with special reference to sewage sludge. Soil Biol. Biochem. 22(7), pp 977-982.

(86) Witter E, Martensson AM, Garcia FV (1993). Size of the soil microbial biomass in a long term field experiment as affected by different N fertilisers and organic manures. Soil Biol. Biochem. 25(6), pp 659-669.

(87) Suwa Y, Imamura Y, Suzuki T, Tashiro T and, Urushigawa Y (1994). Ammonia-Oxidizing bacteria with different sensitivities to (NH4)2SO4 in activated sludges. Wat Res 28(7), 1523-1532.

(88) Sathyanesan AG et al. (1978). Endocrine Changes in fishes in response to pollutants. Quart. J. Surg. Sci. 14, 67-77.

(89) Ram RN and Sathyanesan AG (1986). Ammonium sulfate induced nuclear changes in the oocyte of the fish, Channa punctatus (Bl.). Bull. Environ. Contam. Toxicol. 36, 871-875.

(90) Ram RN and Sathyanesan AG (1987). Effect of chronic exposure of commercial nitrogenous fertilizer, ammonium sulfate, on testicular development of a teleost Channa punctatus (Bloch). Indian J. of Experimental Biology 25, 667-670.

(91) Ram RN and Sathyanesan AG (1986). Inclusion bodies:formation and degeneration of the oocytes in the fish Channa punctatus (Bloch) in response to ammonium sulfate treatment. Ecotoxicology and Environmental Safety 11, 272-276.

(92) Ram RN, Sathyanesan AG (1987). Histopathological Changes in Liver and Thyroid of the Teleost Fish, Channa Punctatus (Bloch) in Response to Ammonium Sulfate Fertilizer Treatment. Ecotoxicology and Environmental Safety 13,185-190.

(93) Abbés C, Parent LE, Karam A, Isfan D (1995). Onion response to ammoniated peat and ammonium sulphate in relation to ammonium toxicity. Canadian J Soil Sci. 75(3), p 261-272.

(94) Gonzalez Ponce R, Salas ML (1989). Effects of different nitrogen compounds and temperatures on the germination of Avena sterilis spp Macrocarpa Mo. Biologia Plantarum (Praha), 31(4), 261-268.

(95) Rosengren-Brinck U, Nihlgard B (1995). Effects of nutritional status on the drought resistance in Norway spruce. Water, Air, and Soil Pollution 85, 1739-1744.

(96) van Hezewijk MJ, Verkleij JAC (1996). The effect of nitrogenous compounds on in vitro germination of Orobanche crenata Forsk. Weed Research 36, 395-404.

(97) Heneghan L, Bolger T (1996). Effects of acid rain components on soil microarthropods: a field manipulation. Pedobiologica 40, 413-438.

(98) Berger H, Foissner W, Adam H (1984). Effects of ammonium sulfate ((NH4)2SO4) on Soil Testacea, Ciliates, and Nematodes. Annual Meeting of the Society of Protozoologists (German Section), Bonn, West Germany. March 29-31, 1984. J Protozool 31(4), p. 59A.

(99) Talavera C, Mendoz M, Moss S (1984). Effect of different sources and doses of nitrogen on damage caused by meloidogyne incognita in tomato lycopersicon-esculentumplants. Agrociencia 0(56), 107-120.

(100) Sibbald IR and Cave NA (1976). The response of chicks to ammonium, calcium, magnesium, potassium and sodium sulfates and to ammonium and potassium carbonates. Poultry Science 55, 2209-2213.

(101) Apgar LA, Meek CL, Geaghan JP (1985). Toxicity of riceland agrichemicals on Thermonactus basillaris (Harris), a predator of mosquito larvae. The Southwestern Entomologist 10(3), 222-227.

(102) Cairney JWG and Meharg AA (1999). Influences of anthropogenic pollution on mycorrhizal fungal communities.Environmental pollution 106, p 169-182.

(103) Umabhaskara Mani V, Venkateswara Rao P, Satyavelu Reddy K, Indira K, Rajendra W (1993). Bioaccumulation and clearance of ambient ammonia by the freshwater mussel, Lamellidens marginalis L. Indian Journal of Comparative Animal Physiology 11(2), p 83-88.

(104) EPA (1978). U.S. Environmental Protection Agency: Studies on the Effect of Ammonium Sulfate on Carcinogenesis. March 1978. EPA-600/1-78-020, National Technical Information Service Publication PB 280 336.

(105) Sato A et al. (1999). A case of fatal intoxication with ammonium sulfate and a toxicological study using rabbits Forensic Science International 101, 141-149.

(106) Roberge A and Charbonneau R (1969). Le metabolisme de l'ammoniaque. III - Effet in vitro de l'arginine sur les enzymes du cycle de l'urée lors de l'intoxication à l'ammoniaque. Rev Can Biol 28(2), 111-118.

(107) Post RL, Jolly PC (1957). The linkage of sodium, potassium and ammonium active transport across the human erythrocyte membrane. Biochem Biophys Acta 25, 118-128,

(108) Conn HO (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. As cited in: WHO (1986). Environmental Health Criteria 54. Ammonia. Published under the joint sponsorhip of the United Nations Environment Programme, the International Labour Organisation and the World Health Organization. Geneva, 1986.

(109) Roberge A and Charbonneau R (1968). Metabolism of Ammonia. I. Biochemical Aspect of Ammonia Intoxication. Rev Can Biol27(4), 321-331.

(110) EPA (2002). U.S. Environmental Protection Agency. Drinking Water Advisory: Consumer Acceptability Advice and Health Effects Analysis on Sulfate (Draft). April 2002.

(111) BASF AG (1969). Department of Toxicology. Unpublished data (XIX/179), 09 September 1969.

(112) Anon (1988). Gigiena i Sanitariya, 53(2), p. 93. As cited in RTECS, update January 2001

(113) Yamanaka S et al. (1990). A simple method for screening assessment of acute toxicity of chemicals. Arch Toxicol 64,262-268.

(114) Frank JF (1948). The toxicity of sodim chlorate herbicides. Can J Comp Med 12, 216-218. As cited in: Yamanaka S et al (1990). A simple method for screening assessment of acute toxicty of chemicals. Arch. Toxicol. 64, 262-268.

(115) Batyrova TF and Astafeva IK (1971). Comparative study of the toxicity of two ammonium sulfate specimens. Gig Tr Prof Zabol 15 (10), 53-54.

(116) Phalen RF, Kenoyer JL, Crocker TT and McClure TR (1980).effects of sulfate aerosols in combination with ozone on elimination of tracer particles inhaled by rats. J. Toxicol. Environm. Health 6, 797-810.

(117) Ehrlich R et al. (1978) Susceptibility to bacterial pneumonia of animals exposed to sulfates. Tox Letters 1, 325-330.

(118) Schlesinger RB (1984). Comparative Irritant Potency of Inhaled Sulfate Aerosols - Effects of Bronchial Mucociliary Clearance. Environmental Research 34, 268-279.

(119) Sackner MA et al. (1981). Effects of brief and intermediate exposures to sulfate submicron aerosols and sulfate injections on cardiopulmonary function of dogs and tracheal mucous velocity of sheep. J. Toxicol. Env. Health 7, 951-972.

(120) Pepelko WE et al. (1980). Toxicology of Ammonium Sulfate in the Lung. Bull. Environm. Contam. Toxicol. 24, 156-160.

(121) Amdur MO Bayles J., Ugro V. and Underhill D.W.(1978). Comparative irritant potency of sulfate salts. Environ Res 16, 1-8.

(122) Frank R et al. (1977). Respiratory Effects of Inhaled Gases and Aerosols. Rept. for 1 Nov 73 - 31 Mar 77. Washington Univ., Seattle.Dept. of

Environmental Health. Jul 77, 57 p.; Govt. Reports Announcements & Index; Iss. 23; NTIS/PB89-227862, (1989)

(123) Schlesinger RB et al. (1978) Effects of short-term exposures to sulfuric acid and ammonium sulfate aerosols upon bronchial airway function in the donkey. Am Ind Hyg Assoc J 39, 275-286.

(124) Abraham WM et al. (1983). Airway Effects of Acid Sulfates in Conscious Sheep. Proceedings of the 76th Annual Meeting of the Air Pollution Control Association Atlanta, June 19-24, 83-8.6, p. 1-12.

(125) University of California (1941). Berkeley, Publications in Pharmacology, Berkeley, CA. Vol. 2, p. 1. As cited by RTECS, update January 2001.

(126) Hayes J.A. and Shiga A. (1969). Ultrastructural changes in pulmonary oedema produced experimentally with ammonium sulphate. J. Path. 100, 281-286.

127) BASF AG (1968). Department of Toxicology. Unpublished data (XVII/352-363), 24 January 1968.

(128) Busch R.H., Buschbom R.L. and Smith L.G.(1984). Comparison of methods for evaluation of experimentally induced emphysema. Environ. Res. 33, 473-496.

(129) Busch RH, Buschbom RL, Cannon WC, Lauhala KE, Miller fJ,Graham JA, Smith LG (1984). Effects of Ammonium Sulfate Aerosol Exposure on Lung Structure of Normal and Elastase-Impaired Rats and Guinea Pigs. Environmental Research 33(2), 454-472.

(130) Smith LG, Busch RH, Buschbom RL, Cannon WC, Loscutoff SM, Morris JE (1989). Effects of sulfur dioxide or ammonium sulfate exposure, alone or combined, for 4 or 8 months on normal and elastase-impaired rats. Environmental Research 49(1), 60-78.

(131) Warren DL et al. (1986). Toxicology and Applied Pharmacology 84(3), 470-479.

(132) Loscutoff SM, Cannon WC, Buschbom RL, Busch RH, Killand BW (1985). Pulmonary function in elastase-treated guinea pigs and rats exposed to ammonium sulfate or ammonium nitrate aerosols. Environmental Research 36, 170-180.

(133) Kleinman MT, Bhalla DK, Mautz WJ, and Phalen RF (1995). Cellular and Immunologic Injury with PM-10 Inhalation. Inhalation Toxicology 7, 589-602.

(134) Takagi H, Onodera H, Yun L, Yasuhara K, Koujitani T, Mitsumori K, Hirose M (1999). 13-Week Subchronic Oral Toxicity Study of Ammonium Sulfate in Rats. Bull Natl Health Sci 117, 108-114 [in Japanese].

(135) Tsunoda M and Sharma RP (1999). Modulation of tumor necrosis factor alpha expression in mouse brain after exposure to aluminum in drinking water. Arch Toxicol 73, 419-426.

(136) Fazekas IG (1954). Vergrösserung der Nebenschilddrüsen durch einfache acidotische Verbindungen. Virchows Archiv 324, 531-542 [in German].

(137) Godleski JJ, Melnicoff MJ, Sadri S, Garbell P (1984). Effects of inhaled ammonium sulfate on benzo(a)pyrene carcinogenesis. J. Toxic. Environm. Health 14, 225-238.

(138) BASF AG (1989). Department of Toxicology. Unpublished report (88/736). 26 June 1989.

(139) Litton Bionetics (1975). Mutagenic evaluation of compound FDA 73-42: Ammonium sulfate granular food grade. Submitted to the US Food and Drug Administration, 30 June 1975. National Technical Information Service PB 245-506.

(140) Obe G, Jonas R, Schmidt S (1986). The restriction endonuclease Alu I induces chromosomal aberrations in human peripheral lymphocytes in vitro. Mutation Research 163, 271-275.

(141) Tuschy S and Obe G (1988). Potentiation of Alu I-induced chromosome aberrations by high salt concentrations in Chinese hamster ovary cells. Mutation Research 207, 83-87.

(142) Obe G and Kamra OP (1986). Elevation of Alu I-induced frequencies of chromosomal aberrations in Chinese hamster ovary cells by Neurospora crassa endonuclease and by ammonium sulfate. Mutation Research 174, 35-46.

(143) Nowak C (1987). Studies on the ability of hypotonic solutions to induce chromosomal aberrations in V79 cells Teratogenesis, Carcinogenesis, and Mutagenesis 7, 515-525.

(144) Sirover MA and Loeb LA (1976). Infidelity of DNA Synthesis in vitro: Screening for Potential Metal Mutagens or Carcinogens. Science 194, 1434-1436.

(145) Nowak C (1988). Influence of ammonium sulfate and sodium chloride on ethyl methanesulfonate-induced chromosomal aberrations and HPRT mutatins in V79 hamster cells. Mutation Research 207, 147-152.

(146) Hayashi M et al. (1988). Micronucleus test in mice on 39 food additives and eight miscellaneous chemicals. Fd. Chem. Toxic 26, 487-500.

(147) Furihata C et al. (1989). Various Sodium Salts, Potassium Salts, a Calcium Salt and an Ammonium Salt Induced Ornithine Decarboxylase and Stimulated DNA Synthesis in Rat stomach Mucosa. Jpn J Cancer Res 80, 424-429.

(148) EPA (1988). Genetox Program. As cited in RTECS, update January 2001.

(149) Andres CJ, Cline TR (1989). Influence of sulfate in drinking water on mouse reproduction during two parties. J Anim Sci 67, 1313-1317.

(150) Fazekas IG (1949). [Experimental data on influencing ovarian functions by simple compounds]. Orv Hetil 90, 777-781 [in German]. As cited in WHO (1986) and WHO (1996).

(151) The Fertilizer Institute (2002). Unpublished data. DAP 4-Week General Toxicity and Reproductive/Developmental Toxicity Screening Test. WBG 001/022948. 20 November 2002

(152) Seidenberg JM, Anderson DG, Becker RA (1986). Validation of an in Vivo Developmental Toxicity Screen in the Mouse. Teratogenesis, Carcinogenesis, and Mutagenesis 6, 361-374.

(153) Verret MJ et al. (1980). Toxicity and teratogenicity of food additive chemicals in the developing chicken embryo Toxicol. Appl. Pharmacol. 56, 265-273.

(154) Paterson DW et al. (1979). Effects of sulfate in water on swine reproduction and young pig performance. J Anim Sci 49, 664-667.

(155) Lake BG et al. (1979). The Effect of Ammonium Sulfate on the Metabolism of Dimethylnitrosamine and Other Xenobiotics By Rat Hepatic Microsomes. Drug Metab Dispos 7(3), 181-187.

(156) Roberge A et al. (1969). Le metabolisme de l'ammoniaque. IV. Effet in vivo d'injections prolongées de sulfate d'ammonium et d'arginine aur l'activité spécifique des enzymes du cycle del'urée.Rev Can Biol 28(2), 119-126.

157) Clarke ML et al. (1981). Veterinary Toxicology, 2nd ed.,London, Bailliere Tindall, p. 26. As cited in HSDB, 04/2001.

(158) European Commission (2000). Ammonium Sulphate. InternationalUniform Chemical Information Database. Year 2000 CD-ROMedition.

(159) Bowley R, pachington A. (1994). Polioencephalomalacia associated with the ingestion of ammonium sulfate. The Veterinary Record june 11, 636.

160) Jeffrey M. et al. (1994). Polioencephalomalacia associated with the ingestion of ammonium sulfate by sheep and cattle. The Veterinary Record 134, 343-348.

(161) Huston J.E. and Eng K.S.(1974). Comparative effects of ammonium sulfate and urea on toxicity, blood ammonia and blood urea in sheep. Feedstuffs, June 2, 22-23.

(162) Hertl M.C. et al (1994). The effect of ammonium sulfate injection on peripheral nerves in rats of varying ages. Neurologic surgery 45, 603-608.

(163) Singer H.R.et al. (1971). Pathology changes resulting from acute ammonium salt poisoning. Am. J.Vet. Res. 32 (8)1239-1246.

(164) Last J. A. et al.(1984). A mechanism of synergistic lung damage by ozone and a respirable aerosol. Exp. Lung Res. 7, 223-235.

(165) Last J.A. (1983). Health effects of indoor air pollution: syergistic effects of nitrogen dioxide and a respirable aerosol. Environment International 9, 319-322.

(166) Last J.A. et al. (1983). Synergistic effects on rat lungs of mixtures of oxidant air pollutants (ozone or nitrogen dioxide) and respirable Aerosols. Am Rev. Respir. Dis. 128, (3), 539-544.

(167) Last J.A. et al. (1986). Synergistic interaction of opzone and respirable aerosols in rat lungs. I. Importance of earosol acidity

(168) Richards R.J.et al. (1990). Clara cell culture from the mouse and their reaction to bronchiolar toxins. Environm. health Perps. 85, 119-127.

(169) Schlesinger R.B (1989), Factors affecting the response to acid aerosols: role of exposure concentration, exposure time, and relative acidity. Environm. Health Perspectives 79, 121-126.

(170) Kfir R and Prozesky OW (1981). Detection of toxic substances in water by means of a mammalian cell culture technique. Water Res. 15, 553-559.

(171) Arany C et al. (1983). Effects of subchronic exposure to a mixture of 03,SO2, and (NH4)2SO4 on host defenses of mice. J.Toxicol. environm. Health 12, 55-71.

(172) Kleinmann M.T. et al. (1985). Comparative effects of acid and non-acidic multicomponent atmospheres on the lungs of rats exposed by inhalation. Proceedings of Air Poll. Contr. Ass. 3, 1-10.

(173) Utell MJ, Morrow PE, Speers DM, Darling J, Hyde RW (1983). Airway responses to sulfate and sulfuric acid aerosols in asthmatics: An exposure-response relationship. Am Rev Respir Dis 128(3), 444-450.

(174) Stacy RW, Seal Jr E, House DE, Green J, Roger LJ, Raggio L (1983). A survey of effects of gaseous and aerosol pollutants on pulmonary function of normal males. Archives of Environmental Health 38, 104-115.

(175) Avol EL, Jones MP, Bailey RM, Chang N-MN, Kleinman MT, Linn WS, Bell KA, Hackney JD (1979). Controlled Exposures Of Human Volunteers To Sulfate Aerosols Health Effects And Aerosol Characterization. American Review of Respiratory Disease, Vol. 120, 319-327.

(176) Swaen GM, Slangen JMM, Ott MG, Kusters E, Van Den Langenbergh G, Arends JW, Zober A (1996). Investigation of a cluster of ten cases of Hodgkin`s disease in an occupational setting. Int Arch Occup Environ Health 68, 224-228.

(177) Kulle TJ et al. (1984). Sulfur dioxide and ammonium sulfate effects on pulmonary function and bronchial reactivity in human subjects. Am. Ind. Hyg. Assoc. J. 45, 156-161.

(178) Belanger PL (1986). Health Hazard Evaluation Report No HETA-85-029-1675, JR Simplot Company, Helm, California. March 1986.

(179) Yamaguchi N et al. (1992). A Case-Control Study on Occupational Lung Cancer Risks in an Industrialized City of Japan. Japanese Journal of Cancer Research (Gann) 83, 134-140.

(180) Okropiridze G (1977). A case of poisoning by ammonium sulfate in drinking water. Gig. Sanit. 2, 100 (in Russian).

(181) Utell MJ, Morrow PE, Hyde RW (1982). Comparison of normal and asthmatic subjects` responses to sulphate pollutant aerosols. Ann Occup Hyg 26(1-4), 691-697.

(182) EPA/OTS (1997). Doc# 88970000213, BioLab Inc.

(183) Anon (1986). Indian Chemicals Manufacturers Association Information Sheet, Calcutta.

(184) Koenig J. Q., Dumler K., Rebolledo V., et al., (1993). Arch. Environ. Health, 48, 171-175

(185) Ericsson G and Camner P (1983). Health effects of sulfur oxides and particulate matter in ambient air. Scand J Work Environ Health 9, Suppl 3, 3-52.

(186) BASF AG (2004) Workplace measurements according GefSoffV. 18, 24/25.June 2004), Project. No: DE/LU/L515/0267/04, 30. June 2004