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# Provisional Peer Reviewed Toxicity Values for

# Ammonia

(CASRN 7664-41-7)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
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## Acronyms and Abbreviations

| | |
|---|---|
| bw | body weight |
| cc | cubic centimeters |
| CD | Caesarean Delivered |
| CERCLA | Comprehensive Environmental Response, Compensation and Liability Act of 1980 |
| CNS | central nervous system |
| cu.m | cubic meter |
| DWEL | Drinking Water Equivalent Level |
| FEL | frank-effect level |
| FIFRA | Federal Insecticide, Fungicide, and Rodenticide Act |
| g | grams |
| GI | gastrointestinal |
| HEC | human equivalent concentration |
| Hgb | hemoglobin |
| i.m. | intramuscular |
| i.p. | intraperitoneal |
| i.v. | intravenous |
| IRIS | Integrated Risk Information System |
| IUR | inhalation unit risk |
| kg | kilogram |
| L | liter |
| LEL | lowest-effect level |
| LOAEL | lowest-observed-adverse-effect level |
| LOAEL(ADJ) | LOAEL adjusted to continuous exposure duration |
| LOAEL(HEC) | LOAEL adjusted for dosimetric differences across species to a human |
| m | meter |
| MCL | maximum contaminant level |
| MCLG | maximum contaminant level goal |
| MF | modifying factor |
| mg | milligram |
| mg/kg | milligrams per kilogram |
| mg/L | milligrams per liter |
| MRL | minimal risk level |

| | |
|---|---|
| MTD | maximum tolerated dose |
| MTL | median threshold limit |
| NAAQS | National Ambient Air Quality Standards |
| NOAEL | no-observed-adverse-effect level |
| NOAEL(ADJ) | NOAEL adjusted to continuous exposure duration |
| NOAEL(HEC) | NOAEL adjusted for dosimetric differences across species to a human |
| NOEL | no-observed-effect level |
| OSF | oral slope factor |
| **p-IUR** | **provisional inhalation unit risk** |
| **p-OSF** | **provisional oral slope factor** |
| **p-RfC** | **provisional inhalation reference concentration** |
| **p-RfD** | **provisional oral reference dose** |
| PBPK | physiologically based pharmacokinetic |
| ppb | parts per billion |
| ppm | parts per million |
| **PPRTV** | **Provisional Peer Reviewed Toxicity Value** |
| RBC | red blood cell(s) |
| RCRA | Resource Conservation and Recovery Act |
| RDDR | Regional deposited dose ratio (for the indicated lung region) |
| REL | relative exposure level |
| RfC | inhalation reference concentration |
| RfD | oral reference dose |
| RGDR | Regional gas dose ratio (for the indicated lung region) |
| s.c. | subcutaneous |
| SCE | sister chromatid exchange |
| SDWA | Safe Drinking Water Act |
| sq.cm. | square centimeters |
| TSCA | Toxic Substances Control Act |
| UF | uncertainty factor |
| µg | microgram |
| µmol | micromoles |
| VOC | volatile organic compound |

2-2-2005

# PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR AMMONIA (CASRN 7664-41-7)

## Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions (or the EPA HQ Superfund Program) sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

**Disclaimers**

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

**Questions Regarding PPRTVs**

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

## INTRODUCTION

The HEAST (U.S. EPA, 1997) lists subchronic and chronic oral reference doses (RfDs) of 34 mg/L for ammonia. A comment in the HEAST indicates that 34 mg/L is a concentration in drinking water that is specifically related to the organoleptic (taste) threshold and that a safe concentration for ammonia may be higher than 34 mg/L, but the data are inadequate to assess the safe level. The source document for derivation of the HEAST subchronic and chronic oral RfD values is the Health Effects Assessment (HEA) for Ammonia (U.S. EPA, 1987). The HEAST subchronic and chronic RfD values are based on a determination of the organoleptic (taste) threshold of ammonia in redistilled water by Campbell et al. (1958). The value selected for the HEAST subchronic and chronic RfDs was supported by the closely similar value of 35 mg/L identified as the taste threshold for ammonia in a World Health Organization Environmental Health Criteria (EHC) document (WHO, 1986) and as the ambient water quality criterion to

protect human health derived by U.S. EPA (1981). No oral assessment is included on IRIS (U.S. EPA, 2003) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2002). No relevant documents other than the HEA and the AWQC document were included in the CARA list (U.S. EPA, 1991, 1994a).

The HEAST includes a value of 1E-1 $mg/m^3$ for the subchronic inhalation RfC. The HEAST subchronic RfC used the same data and is the same as the RfC (1E-1 $mg/m^3$) reported in IRIS (U.S. EPA, 2003). The IRIS chronic RfC value was derived from a free-standing NOAEL of 6.4 $mg/m^3$ (9.2 ppm) identified for lack of evidence of decreased pulmonary function or changes in subjective symptomatology in an occupational study of workers exposed to ammonia in a soda ash (sodium carbonate) facility (Holness et al., 1989). A LOAEL was not identified in the study. The NOAEL was adjusted for intermittent exposure to a value of 2.3 $mg/m^3$ and divided by a composite uncertainty factor (UF) of 30. The composite UF included a factor of 10 for protection of sensitive individuals and a factor of 3 for database deficiencies, including lack of chronic data, proximity of the occupational $NOAEL_{HEC}$ to a $LOAEL_{HEC}$ observed in a subchronic inhalation study in rats (Broderson et al., 1976), and lack of data on reproductive or developmental toxicity. The RfD/RfC Workgroup verified the RfC on February 21, 1991. The HEA had previously derived subchronic and chronic inhalation RfDs of 0.36 $mg/m^3$ by dividing the ammonia air odor threshold of 3.6 $mg/m^3$ (Carson et al., 1981) by an uncertainty factor of 10 to obtain an estimate of the lower bound limit for odor detection.

The public review draft of the ATSDR Toxicological Profile on ammonia (ATSDR, 2002) derived an intermediate oral minimal risk level (MRL) value of 0.3 mg/kg-day based on a duration-adjusted NOAEL of 39.5 mg/kg-day for weight loss in rats exposed to ammonium sulfamate in drinking water for 90 days (Gupta et al., 1979) and an uncertainty factor of 100 (10 for extrapolation from rats to humans and 10 to protect sensitive individuals). ATSDR (2002) also derived a chronic inhalation MRL of 0.3 ppm (200 $\mu g/m^3$) based on a duration-adjusted NOAEL of 3.1 ppm in the Holness et al. (1989) study and an uncertainty factor of 10 for human variability. The State of California (OEHHA, 2002) has derived a chronic inhalation reference exposure level of 200 $\mu g/m^3$ (0.3 ppm) for ammonia. This value (200 $\mu g/m^3$) is based on the occupational study of Holness et al. (1989) with a duration adjusted NOAEL of 2 $mg/m^3$ and an uncertainty factor of 10 for intraspecies variability. The OEHHA (2002) used the same methodology as ATSDR. ACGIH (2001) lists a TLV-TWA of 25 ppm (17 $mg/m^3$) and a STEL of 35 ppm (24 $mg/m^3$) for ammonia. These values are intended to minimize the potential for acute ocular and respiratory tract irritation. NIOSH (2002) lists values of 25 ppm (18 $mg/m^3$) and 35 ppm (27 $mg/m^3$) for the REL-TWA and REL-ST, respectively. OSHA (2002) lists a value of 50 ppm (35 $mg/m^3$) for the PEL-TWA.

Ammonia is not included in the HEAST (U.S. EPA, 1997) cancer table. IRIS (U.S. EPA, 2003) and the Drinking Water Standards and Health Advisories list (U.S. EPA, 2002) do not provide a carcinogenicity assessment for ammonia. IARC (2002) has not evaluated the

carcinogenicity of ammonia. NTP (2002) does not list ammonia among the chemicals it considers to be known human carcinogens or reasonably anticipated to be human carcinogens.

Literature searches to identify studies relevant to the derivation of provisional toxicity values for ammonia were conducted for the period 1988 through September 18, 2002. Databases searched included: TOXLINE, MEDLINE, TSCATS, RTECS, CCRIS, DART, EMIC/EMICBACK, HSDB, GENETOX and CANCERLIT. Additional literature searches were conducted through May 2004 by NCEA-Cincinnati using TOXLINE, MEDLINE, Chemical and Biological Abstract databases and no relevant information was found.

## REVIEW OF PERTINENT DATA

### Human Studies

Holness et al. (1989) studied workers exposed to ammonia in a sodium carbonate production plant. Fifty-two of the 64 available workers agreed to participate in the study. The control group consisted of 31 office and stores workers employed at the plant who were without previous exposure to ammonia. Information was collected on age, height, work history, smoking history, respiratory symptoms, and skin and eye complaints. Respiratory questions were based on an American Thoracic Society questionnaire. Sense of smell was evaluated at the beginning and end of the work week. Pulmonary function tests were performed at the beginning and end of each work shift on two test days. The parameters measured were forced vital capacity (FVC), forced expiratory volume in one second ($FEV_1$); and forced expiratory flow rate at 50% and 75% of the vital capacity ($FEF_{50}$ and $FEF_{75}$). Mean time-weighted average (TWA) exposures to ammonia were determined by personal air sampling over one shift following NIOSH recommendations. The average sampling time was 8.4 hours. The mean age of the exposed workers was 38.9 ± 11.7 years and the average duration of exposure was 12.2 ± 8.9 years. Only weight differed significantly when demographics for the exposed and control workers were compared. Time-weighted average airborne concentrations of ammonia were 9.2 ± 1.4 ppm (6.4 $mg/m^3$) and 0.3 ± 0.1 ppm (0.2 $mg/m^3$) for the exposed and control groups, respectively. Although no significant difference was evident between exposed and control groups in reporting of respiratory symptoms, workers reported that exposure at the plant aggravated specific symptoms including coughing, wheezing, nasal complaints, eye irritation, throat discomfort, and skin problems. No significant differences were evident between the exposed and control groups in reporting of respiratory symptoms, sense of smell, baseline lung function, or change in lung function at the beginning and end of a work week. No significant relationships between level or length of ammonia exposure and lung function results were demonstrated. The NOAEL in this study was 9.2 ppm (6.4 $mg/m^3$), based on lack of evidence for decreased pulmonary function or changes in subjective assessments of respiratory symptoms. A LOAEL was not identified in this study.

Ferguson et al. (1977) exposed healthy human volunteers (2/concentration) employed in an alkali plant to ammonia concentration of 25 ppm, 50 ppm, or 100 ppm, 5 days/week for six weeks. Conclusions of the study were actually based on 5 weeks of exposure as a result of technical difficulties during the first week of the study. Toxicity was assessed by subjective and objective indications of eye and respiratory tract irritation, pulse rate, respiration rate, pulmonary function (FVC, FEV), physical examination, and the ability to perform routine tasks. Exposure to ammonia did not result in abnormalities of the chest, heart, vital organs, neurological response, task performance or significant weight changes as assessed during weekly medical examinations. Transient irritation of the throat was observed at exposures of 50 ppm (4 hours/day).

More recently published occupational studies were examined to identify data potentially suitable for calculation of a subchronic RfC. Ballal et al. (1998) reported the results of a cross-sectional study of male workers employed in two fertilizer plants in Saudi Arabia. Exposure to ammonia concentrations of 25 ppm and above were significantly associated with respiratory symptoms including wheezing, cough, phlegm, dyspnea, and asthma. Ali et al. (2001) examined the pulmonary function of workers (gender not specified) in an ammonia-producing factory in Saudi Arabia. Cumulative exposure of greater than 50 $mg/m^3$-years was associated with significantly reduced $FEV_1$ and FVC. Symptomatic workers (i.e., those reporting cough, phlegm, wheeze, and/or dyspnea) showed significantly reduced $FEV_1$and $FEV_1$/FVC ratio when compared to asymptomatic workers. Neither study adequately reported details of worker exposure, such as the number of hours worked per week, and were not further considered for derivation of reference doses.

A number of studies have examined the relationship between inhalation exposure to pollutants (including ammonia) in livestock confinement buildings and occurrence of respiratory symptoms and/or changes in pulmonary function in workers (Heerderik et al., 1990; Choudat et al., 1994; Donham et al., 1995, 2000; Reynolds et al., 1996; Vogelzang et al. 1997, 2000; Cormier et al., 2000). Exposure to ammonia concentrations of 2.3 to 20.7 ppm was associated with symptoms of bronchial reactivity, inflammation, cough, wheezing, or shortness of breath and decrements in pulmonary function as measured by $FEV_1$, maximum expiratory flow rate, and maximal mid-expiratory flow rate. These data are of limited use for derivation of a subchronic toxicity reference value for ammonia because workers were concurrently exposed to other potential respiratory toxicants such as dusts, endotoxins, and nitrogen dioxide.

The carcinogenic potential of ammonia via the inhalation route has not been assessed in humans.

The experimental database for human oral exposure to ammonia consists of acute and short-term studies of exposure to ammonium chloride. No subchronic or chronic duration oral exposure studies were located in the literature examined. The availability of studies on ammonium chloride is a result of its use for experimental induction of hyperchloremic metabolic

acidosis. Few of the available studies have been designed or conducted to specifically assess the toxicity of ammonia or ammonium ion in response to oral dosing.

U.S. EPA (1981) reviewed fifteen existing short-term studies of ammonium chloride in humans. Administration of ammonium chloride to all age groups produced metabolic acidosis, with increased susceptibility observed in infants. The results of these studies indicate that metabolic acidosis, impaired glucose tolerance, and reduced tissue sensitivity to insulin may result from doses of ammonium chloride greater than or equal to 100 mg/kg-day (31.8 mg ammonia/kg-day, as estimated by U.S. EPA, 1981). Although frank toxicity was not reported, U.S. EPA (1981) expressed concern for potential bone demineralization as a result of impaired acid-base balance.

In the longest duration human study found, Lemann et al. (1966) investigated the electrolyte balance of five men who were given doses of ammonium chloride to induce metabolic acidosis. Each individual served as his own control. Following baseline observations, each subject was given a small initial dose which was progressively increased over a period of six to nine days, after which the dose remained constant until administration of ammonium chloride was discontinued after day 18. U.S. EPA (1987) reported total doses of 733 mEq (approximately 93 mg/kg-day) for the initial loading period and 2771 mEq (approximately 177 mg/kg-day) for the remainder of the experiment. During ammonium chloride loading, net fixed acid production was increased by an average of 3425 mEq. Progressive acid retention was initially accompanied by a progressive decrease in serum bicarbonate concentration. Serum bicarbonate levels dropped as acid was retained during the first nine days of ammonium chloride dosing, stabilized at a reduced level by about day 12, and rose slightly between days 13 to 18, but did not return to baseline levels until after treatment with ammonium chloride was discontinued. Calcium and phosphorus balances became negative as a result of urinary losses, suggesting to the study authors that slow dissolution of bone mineral was occurring to provide additional buffering capacity. The LOAEL in this study was the initial dose of 93 mg/kg-day.

The carcinogenic potential of ammonia via the oral route has not been assessed in adequately designed epidemiological studies.

**Animal Studies**

Broderson et al. (1976) continuously exposed F344 rats (6 rats/sex/dose) to ammonia concentrations of 25, 50, 150, or 250 ppm for seven days prior to inoculation with *Mycoplasma pulmonaris* and for 28 to 42 days following inoculation. These exposures were conducted using purified ammonia from a commercial source. In addition, one treatment group was exposed to ammonia produced from a natural source (soiled bedding) for 30 days following inoculation. Each treatment group had a corresponding control group that was inoculated with *M. pulmonaris* and exposed only to background levels of ammonia. Additional groups were exposed to

background or high levels of ammonia (trace and 250 ppm, respectively) without *M. pulmonaris* inoculation. Toxicity was assessed by observation of clinical signs and histopathological examination of nasal passages, middle ear, trachea, lungs, liver, kidney, adrenal, pancreas, testicle, spleen, mediastinal nodes, and thymus. Clinical signs were similar in control and exposed groups during the pre-inoculation exposure period. Signs of murine respiratory mycoplasmosis (MRM) were observed in all groups approximately 10 days after inoculation. All levels of ammonia from bedding or the commercial source increased the severity of the rhinitis, otitis media, tracheitis, and pneumonia characteristic of MRM. The prevalence and extent of gross atelectasis and consolidation were greater in rats exposed to high ammonia concentrations (i.e., ammonia concentrations greater than background) and the prevalence of microscopic respiratory lesions was also greater. The prevalence of gross and microscopic lung lesions differed significantly from controls when data from all high exposure groups were summed and compared with pooled control data. Regression analysis indicated a positive relationship between ammonia concentration and prevalence of gross or microscopic lesions. Exposure of uninoculated rats to ammonia resulted in lesions that were unlike those of MRM and which were restricted to the nasal passages. A LOAEL of 25 ppm (17.4 $mg/m^3$) was identified in this study.

Schoeb et al. (1982) inoculated pathogen-free F344 rats with M. pulmonis and exposed groups to trace or 100 ppm (70 $mg/m^3$) concentrations of ammonia for up to 28 days. Growth of *M. pulmonis* was greater in ammonia-exposed rats than in controls and serum immunoglobulin response to the inoculum was also greater in the exposed population. Results of an experiment conducted in rats with cannulated tracheas demonstrated that the nasal passages absorbed virtually all ammonia at administered concentrations of 500 ppm (348 $mg/m^3$) or below.

Coon et al. (1970) continuously exposed male and female Sprague-Dawley and Long Evans rats for a minimum of 90 days to ammonia concentrations of 0, 40, 127, 262, 455, or 470 $mg/m^3$. A LOAEL of 262 $mg/m^3$ was identified on the basis of nasal discharge in 25% of the rats and nonspecific degenerative and circulatory changes in the lungs and kidneys. The upper respiratory tract was not examined for microscopic lesions. In another series of experiments, Coon et al. (1970) exposed rats, guinea pigs, rabbits, dogs and monkeys to ammonia concentrations of 0, 155, or 770 $mg/m^3$ for 8 hours/day, 5 days/week for a total of 30 exposures. This study identified a LOAEL of 770 $mg/m^3$ for lung inflammation in rats and guinea pigs and ocular and nasal irritation in dogs and rabbits. The upper respiratory tract was not examined for presence of lesions.

Anderson et al. (1964) conducted a series of experiments that included continuous exposure of guinea pigs and Swiss albino mice to 20 ppm (13.9 $mg/m^3$) ammonia for up to six weeks and exposure of Leghorn chickens for up to 12 weeks. A separate group of guinea pigs was exposed to 50 ppm (35 $mg/m^3$) ammonia for six weeks. Although no effects were observed after exposure to 20 ppm for four weeks, gross lesions including edema, congestion, and hemorrhage were observed in the lungs of all three species after six weeks. Grossly enlarged and

congested spleens, congested livers and lungs, and pulmonary edema were observed in guinea pigs exposed to 50 ppm ammonia for six weeks.

Weatherby (1952) exposed guinea pigs to 0 or 170 ppm (118 mg/$m^3$) 6 hours/day, 5 days/week for up to 18 weeks. No adverse effects were observed in animals exposed for 6 to 12 weeks. Mild changes were observed in the spleen, kidney suprarenal glands, and liver at 18 weeks. No effects on the lungs were observed. The upper respiratory tract was not examined for lesions.

No effects on ovarian or uterine weights were observed in pigs exposed by inhalation to approximately 5 or 35 ppm ammonia for 6 weeks (Diekman et al., 1993). Continuous exposure of female pigs to approximately 35 ppm ammonia from 6 weeks prior to breeding through gestation day 30 did not significantly affect age to puberty, number of live fetuses, fetus-to-corpus luteum ratio, or fetal length when compared to females exposed to 7 ppm ammonia for the same duration (Diekman et al., 1993).

Limited subchronic and chronic toxicity data are available for ammonia. In an early study, Seegal (1927) administered ammonium chloride doses of 0 or 372 mg/kg-day to rabbits by gavage for 36 days and observed episodes of severe metabolic acidosis and epithelial degeneration in the renal tubules. Similar effects plus softening of the teeth, skull, and ribs were observed at a dose of 234 mg/kg-day given by gavage for 11 months.

Freedman and Beeson (1961) exposed 12 adult male Sprague-Dawley rats to 1.6% ammonium chloride in the drinking water for periods of up to three weeks to evaluate effects on the kidney. Six control animals were provided with tap water. An additional group of 10 rats was given drinking water containing 1% ammonium chloride for an additional 2.5 months to assess subchronic effects. No abnormalities were detected by urinalysis, gross pathology or histologic examination. Physiological adaptation to metabolic acidosis was indicated by increased glutaminase activity per gram of kidney with duration of treatment. No data on water consumption or body weight of the test animals were provided. Assuming that the rats weighed 250 grams and consumed 25 mL of drinking water per day, U.S. EPA (1987) estimated a time-weighted average dose of approximately 360 mg/kg-day.

Gupta et al. (1979) conducted a subchronic exposure study in adult female and weanling male and female ITRC rats (20/sex/age/dose). The test animals were treated with ammonium sulfamate ($NH_4SO_3NH_2$) at doses of 0, 100, 250, or 500 mg/kg-day, 6 days per week for 30, 60, or 90 days. The ammonium sulfamate was given as a 10% solution, but the study report did not clearly indicate whether the dose was administered by gavage. Food and water consumption, appearance, behavior, and body weight were monitored during the study. Hematological parameters and organ weights were measured at interim and terminal sacrifices and tissue samples were collected for histopathological examination. Food and water consumption were

decreased in male and female weanlings at the 500 mg/kg-day dose relative to the controls. No compound-related clinical signs of toxicity were observed in dosed rats. Body weight of adult females receiving 500 mg/kg-day was significantly reduced at 60 day (9%) and 90 days (16%) when compared to the control group. No significant differences were noted in hematological parameters, organ weights, or histopathology. Although the study authors indicated that ammonium sulfamate would be expected (on the basis of its structure) to cause metabolic acidosis, this prediction does not appear to have been confirmed experimentally. These data identify NOAEL and LOAEL values of 250 and 500 mg/kg-day as ammonium sulfamate, respectively. The effective dose of ammonia at each of these dose levels is uncertain, because under certain conditions the sulfamate ion is hydrolyzed to bisulfate ion and ammonia (U.S. EPA, 1981, 1987). Assuming no hydrolysis of the sulfamate ion, these doses correspond to 37.3 and 74.8 mg/kg-day of ammonia, respectively.

Bodega et al. (1993) fed diets containing 0 or 20% ammonium acetate to pathogen-free female Wistar rats (5 rats/group) for 3, 7, 15, 45, or 90 days to assess effects on glial fibrillary acidic protein (GFAP) in the spinal cord. The ammonium acetate in the diet was supplemented by addition of 5 mM ammonium acetate to the drinking water. The total exposure from the combined food and water was not provided by the author. Exposure to ammonium acetate had no effect on behavior, water consumption, or spinal GFAP levels of the test animals. Body weight gain was significantly reduced in dosed animals at all time points. Body weight gain in animals exposed to ammonia for 90 days was 69% of the control value.

Fazekas (1939, 1954a,b) conducted studies in rabbits that ranged from 3 to 17 months in duration. The administration of various ammonium salts (carbonate, chloride, sulfate, hydrophosphate, acetate, or lactate) or ammonium hydroxide resulted in enlargement of the parathyroids. Similar results were obtained with a variety of other chemicals (sodium dihydrophosphate, sodium ammonium phosphate, calcium chloride, hydrochloric acid, acetic acid, lactic acid) (Fazekas, 1954a). The chemicals were given for three week periods separated by one week intervals. The administered dose of ammonium salts in this study is unclear, but based on descriptions in secondary sources is likely to be less than or equal to 0.4 mg/kg-day. In a related study, similar treatment of rabbits with ammonium chloride or ammonium sulfate resulted in fluctuations in serum calcium and phosphorus levels (Fazekas, 1954b). Rabbits given gavage doses of 100 mg/kg by gavage on alternate days and then daily for 17 months developed enlarged adrenal glands. An initial fall in blood pressure of 20 to 30 mg Hg was followed by a gradual rise to levels 10 to 30 mg Hg after several months of treatment.

In a chronic study, Barzel and Jowsey (1969) exposed male Sprague-Dawley rats to 1.5% ammonium chloride in the drinking water for 330 days. The effects of ammonium on animals receiving a nutritionally complete diet included decreased bone content of fat-free solid and calcium; decreased body weight and body fat; and decreased blood pH and plasma carbon dioxide. Barzel (1975) reported effects on bone (decreased density, ash weight, and calcium

content), but no effects on growth, in intact and ovariectomized female rats exposed to 1.5% ammonium chloride in the drinking water for 300 days. U.S. EPA (1981) estimated an average daily dose of 1500 mg/kg-day for both studies.

The carcinogenic potential of ammonia has been investigated in an oral bioassay conducted in mice. Toth et al. (1972) exposed male and female Swiss mice (49-50/sex/dose) to 0.1, 0.2, or 0.3% ammonium hydroxide in the drinking water for their lifetime. U.S. EPA (1987) estimated an average daily dose of 565 mg/kg-day at the highest concentration. Male and female C3H mice (40/sex) were exposed to 0.1% ammonium hydroxide in the drinking water for their lifetime. This concentration corresponded to average daily doses of approximately 270 mg/kg-day, respectively, as calculated by U.S. EPA (1987). While data for a control group are reported in the publication, it is not clear whether this group was run concurrently with the ammonia treatment groups. The mice were examined and weighed at weekly intervals. Moribund animals were humanely sacrificed. Complete necropsies were performed on all animals and the liver, kidney, spleen, lung, and organs with gross lesions were processed for histopathological examination. No evidence for carcinogenicity was observed in males or females of either strain.

Two studies have examined the interaction of ammonia with other compounds in the induction of tumors. Uzvölgyi and Bojan (1980) investigated the interaction of ammonia with diethyl pyrocarbonate (DEPC) in induction of lung tumors in CFPL mice (a urethane-sensitive strain). Mice given gavage doses of either ammonia or DEPC alone did not develop lung tumors, whereas development of lung tumors was observed in mice dosed with both ammonia and DEPC. Induction of tumors in the sensitive CFPL strain may have resulted from formation of urethane *in vivo* from ammonia and DEPC (Uzvölgyi and Bojan,1985). Tsujii et al. (1995) studied the effect of ammonia on tumor development in male Sprague-Dawley rats pretreated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in the drinking water for 24 weeks and subsequently exposed to drinking water containing 0 or 0.01% ammonia for an additional 24 weeks. Exposure to ammonia significantly increased the incidence, multiplicity, size, and depth of tumors in the glandular stomach and stimulated cell proliferation in the gastric mucosa.

Reproductive and developmental toxicity data on ammonia from animal studies are limited. Treatment of virgin female rabbits with oral doses of various ammonium salts (carbonate, chloride, hydrophosphate, or sulfate) or ammonium hydroxide was associated with enlargement of the ovaries, follicle maturation, and formation of corpora lutea (Fazekas, 1949). Enlargement of the uterus, hypertrophy of the teats, and secretion of milk were also reported in treated rabbits. However, several aspects of this study are poorly documented, including the use of controls and exact method of dose administration.

Minaña et al. (1995) examined the effect of prenatal exposure to 20% ammonium acetate in the diet on NMDA receptor function in Wistar rats. As judged from graphically presented data, offspring of dams treated from day 1 of pregnancy through lactation had body weights at

birth that were comparable to the control group. The body weight of weanlings maintained on a diet containing 20% ammonium acetate was reduced by approximately 27% and 26% in males and females, respectively, at 120 days of age when compared to animals maintained on the control diet. Rats exposed to ammonia during pregnancy and lactation and fed an unsupplemented diet at weaning had a lower growth rate than the controls until day 60, indicating persistent effects of prenatal and lactational exposure to ammonia. Prenatal exposure to ammonia reduced binding of [$^3$H]MK-801 to NMDA receptors in primary cultures of cerebellar neurons by approximately 60%. No data were provided for feed intake in this study; therefore, an average daily dose can not be reliably estimated.

**Other Studies**

Information on the toxicokinetic properties of ammonia have been reviewed and summarized in ATSDR (2002). Inhalation exposure studies in humans show that ammonia dissolves in the mucous of the upper respiratory tract. At low levels of exposure, most inhaled ammonia is retained in the upper respiratory system. As the ammonia concentration increases, the capacity of the upper respiratory system is saturated and a larger percentage is absorbed. Development of nasal and pharyngeal irritation, but not tracheal irritation, following exposure is consistent with retention of inhaled ammonia in the upper respiratory tract. Animal data provide supporting evidence for high nasal retention. Quantitative differences in the amount of ammonia in inhaled and exhaled air suggest that small amounts are absorbed across the nasopharyngeal membranes into the systemic circulation. Limited systemic absorption is also inferred from lack of change in blood nitrogen and urinary-ammonia compounds following exposure. The available evidence suggests that ammonium absorbed via inhalation would be distributed to all body compartments by the blood. Ammonium reaching the tissues would be used in protein synthesis or as a buffer, with excess levels reduced by urinary excretion or conversion in the liver to glutamine and urea. Absorbed ammonia is excreted by the kidneys as urea and urinary ammonium compounds. Bioaccumulation to toxic levels is not expected to occur from chronic inhalation exposure based on the low levels of absorption and existence of multiple effective mechanisms for detoxification and excretion.

Human data indicate that ingested ammonium compounds are readily absorbed. The absorbed ammonium ion is transported via the hepatic portal vein to the liver, where most is metabolized to urea in healthy individuals. Data from animals and humans suggest that little of the ingested compound reaches the systemic circulation as ammonia or ammonium ion. Ingested ammonium compounds are excreted primarily in the urine as urea. Small amounts may be excreted in the sweat or in exhaled air.

Genotoxicity data are available from studies in humans, mice, *Escherichia coli*, *Drosophila melanogaster*, and cultured chick fibroblast cells. Yadav and Kaushik (1997) conducted cytogenetic assays on blood samples collected from 22 workers exposed to ammonia

gas (ambient level = 0.09 mg/m$^3$) during production of nitrogen fertilizers and 42 unexposed staff employed at the same facility. The exposed workers did not show clinical symptoms of ammonia toxicity. The mitotic index, total number of chromosome aberrations (CA), and frequency of sister chromatid exchange (SCE) were significantly increased in the exposed workers as compared to their matched controls. The frequency of CA and SCE increased with the duration of exposure. Concurrent exposure to other compounds such as nitrogen dioxide was not addressed in the study report. The frequency of micronuclei was significantly increased in Swiss albino mice treated with intraperitoneal doses of ammonia ranging from 12.5 to 50 mg/kg as compared to controls (Yadav and Kaushik, 1997). Positive results were obtained for reverse mutation in *E. coli*, but only at levels of ammonia that were cytotoxic (Demerec et al., 1951). Negative results were reported for ammonium sulfate in *Salmonella typhimurium* and *Saccharomyces* (Litton Bionetics, 1975). Positive results were observed for chromosomal aberrations in chick fibroblasts treated with buffered ammonium chloride (Rosenfeld, 1932). Reduced cell division and inhibition of DNA repair were observed in mouse fibroblasts treated with ammonia and/or ammonium chloride (Visek et al., 1972; Capuco, 1977). Lobasov and Smirnov (1934) reported slightly mutagenic activity in *D. melanogaster*. Auerbach and Robson (1947) obtained doubtful, probably negative, results for sex-linked recessive mutations in *D. melanogaster* and reported negative results for dominant lethality.

## DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR AMMONIA

Adequate toxicity data for derivation of provisional subchronic or chronic RfD values are not available. The human experimental database consists primarily of older acute and short-term studies in which ammonium chloride was used to induce metabolic acidosis. The longest duration of exposure among these studies was 18 days. The animal database includes one study (Gupta et al., 1979) that was reasonably well-documented, evaluated appropriate endpoints, and included a histopathological evaluation of potential target tissues. This study was not used to derive p-RfD values for two reasons. First, the test article was ammonium sulfamate, which is hydrolyzed under certain conditions to bisulfate ion and ammonia. It is not known whether hydrolysis occurred when the compound was administered to rats; thus, the actual dose of ammonia/ammonium ion administered to the test animals is uncertain. Second, comparison of the data from this study to results from human studies suggests that health effects may occur in humans at lower concentrations of ammonium salts. Gupta et al. (1979) identified a NOAEL equivalent to 37.3 mg ammonia/kg-day and a LOAEL equivalent to 74.8 mg ammonia/kg-day (assuming no hydrolysis of the sulfamate ion; the actual dose may differ). This LOAEL is higher than the 31.8 mg/kg-day level of concern identified for humans by U.S. EPA (1981) for potential bone demineralization. Route-to-route extrapolation is not feasible for derivation of oral reference values because the toxicokinetic properties of ammonia differ significantly for the oral and inhalation pathways. This evaluation of data adequacy is consistent with previous

assessments conducted by U.S. EPA (1981, 1987), which did not use the existing toxicity data for derivation of reference doses.

Because adequate data are lacking for oral exposure to ammonia, previous determinations of toxicity reference values (U.S. EPA, 1981, 1987, 1997) have used organoleptic (taste) data to estimate acceptable ammonium levels in drinking water at 34-35 mg/L. However, organoleptic (taste) data are not reliable predictors of either toxicity or intake. Furthermore, WHO (1986) has identified several limitations of the "triangle test" methodology used to derive the organoleptic (taste) threshold for ammonia: 1) the definition of the threshold is somewhat arbitrary; 2) McBride & Laing (1979) have reported significant positional bias in using the triangle test to determine taste threshold; and 3) the triangle test is not intended to mimic environmental exposures in which the taste thresholds could be substantially higher. Due to the high uncertainty associated with use of the organoleptic (taste) data for ammonia, no oral subchronic or chronic p-RfD is derived.

## DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR AMMONIA

A **chronic RfC of 1E-1 mg/m$^3$** is listed for ammonia on IRIS (U.S. EPA, 2003) based on lack of evidence of decreased pulmonary function in human workers exposed to an estimated concentration of 6.4 mg/m$^3$ for an average of 12.2 years (Holness et al., 1989). The presence of a chronic RfC on IRIS precludes derivation of a provisional chronic RfC for this chemical.

The occupational study of Holness et al. (1989) and the subchronic study conducted in rats by Broderson et al. (1976) were also considered to be an appropriate basis for derivation of a provisional subchronic RfC. Holness et al. (1989) identified a NOAEL of 9.2 ppm (6.4 mg/m$^3$) for apparent lack of effect on pulmonary function or changes in subjective assessments of symptoms in workers exposed to ammonia for a mean duration of 12.2 years while employed at a sodium carbonate production plant. A LOAEL was not identified in this study. The $NOAEL_{HEC}$ was calculated using the default dosimetric adjustment for human data (U.S. EPA, 1994b), as follows:

$$NOAEL_{ADJ} = 6.4 \text{ mg/m}^3 \times 5\text{days}/7\text{days} = 4.6 \text{ mg/m}^3$$

$$NOAEL_{HEC} = NOAEL_{ADJ} \times (VE_{ho}/VE_h)$$
$$= 4.6 \text{ mg/m}^3 \times (10 \text{ m}^3/20 \text{ m}^3)$$
$$= 2.3 \text{ mg/m}^3$$

where,

$VE_{ho}$ = human occupational default minute volume (10 m$^3$/8 hours; U.S. EPA, 1994b)

$VE_h$ = human ambient default minute volume (20 $m^3$/24 hours; U.S. EPA, 1994b)

A $LOAEL_{HEC}$ was calculated from the rat data of Broderson et al. (1976) for comparison with the human $NOAEL_{HEC}$. These researchers identified a LOAEL of 17.4 mg/$m^3$, the lowest concentration tested, for increased severity of rhinitis and pneumonia (with respiratory lesions) in F344 rats inoculated with *M. pulmonis* and continuously exposed to ammonia. The $LOAEL_{HEC}$ is calculated using the procedure for a respiratory effect of a category 1 gas in the extrathoracic region (U.S. EPA, 1994b) as follows:

$LOAEL_{ADJ}$ = $LOAEL_{OBSERVED}$ = 17.4 mg/$m^3$ (continuous exposure)

$LOAEL_{HEC}$ = $LOAEL_{ADJ}$ x $RGDR_{ET}$

$RDGR_{ET}$ = $(V_E / SA_{ET})_A$ / $(V_E / SA_{ET})_H$
= (0.14 $m^3$/day / 15 $cm^2$) / (20 $m^3$/day/ 200 $cm^2$) = 0.093

$LOAEL_{HEC}$ = 17.4 mg/$m^3$ x 0.093
= 1.62 mg/$m^3$ ≈ 1.6 mg/$m^3$

where:

$RDGR_{ET}$ = regional gas deposition ratio in the extrathoracic region
$V_E$ = ventilation rate ($m^3$/day)
$SA_{ET}$ = surface area of extrathoracic region ($cm^2$)
A, H = subscripts denoting laboratory animal and human, respectively
$(V_E)_A$ = 0.14 $m^3$/day (subchronic, female F344 rats; U.S. EPA, 1988)
$(V_E)_H$ = 20 $m^3$/day (U.S. EPA, 1988)
$(SA_{ET})_A$ = 15 $cm^2$ (U.S. EPA, 1994b)
$(SA_{ET})_H$ = 200 $cm^2$ (U.S. EPA, 1994b)

A **subchronic p-RfC of 0.1 mg/$m^3$ (1E-1 mg/$m^3$)** is derived by applying a composite uncertainty factor of 30 to the human NOAEL of 2.3 mg/$m^3$ (Holness et al., 1989). The composite UF includes a factor of 10 to protect sensitive individuals and a factor of 3 for proximity of the animal LOAEL to the human NOAEL and database limitations, including lack of adequate reproductive and developmental toxicity studies. The UF is applied to the human $NOAEL_{HEC}$ of 2.3 mg/$m^3$, as follows:

subchronic p-RfC = $NOAEL_{HEC}$ / UF
= 2.3 mg/$m^3$ / 30
= 0.1 mg/$m^3$ or 1E-1 mg/$m^3$

The animal LOAEL of 1.62 mg/m$^3$ is in close proximity to the human NOAEL of 2.3 mg/m$^3$; however, the extrathoracic effects observed in the animal study were mild and reversible. Furthermore, the animal $LOAEL_{HEC}$ of 1.6 mg/m$^3$ gives a sixteen-fold comparative ceiling to the p-sRfC of 0.1 mg/m$^3$. This adds confidence to the human NOAEL. Thus, the human NOAEL is considered for the derviation of this subchronic p-RfC. Thus the subchronic p-RfC, based on pharmacokinetics, remained the same as the RfC.

Confidence in the principal study is medium because the study was conducted in humans (but the sample size was relatively small), data were collected on males only, and a LOAEL was not identified. Although complaints of exacerbated upper respiratory symptoms were recorded in the principal study and support the extrathoracic region as the critical region for effects, an objective assessment of the workers' nasal epithelium was not performed. However, the observation of mild extrathoracic effects in animals at a HEC similar to the NOAEL support the human findings.

Confidence in the database is medium. The developmental, reproductive, and chronic toxicity of ammonia have not been tested, but toxicokinetic data suggest that ammonia is absorbed by the nasal passages at concentrations comparable to the $NOAEL_{HEC}$ and systemic distribution is unlikely (U.S. EPA, 2003). Medium confidence in the subchronic p-RfC follows.

## DERIVATION OF A PROVISIONAL CARCINOGENICITY ASSESSMENT FOR AMMONIA

Human data on the carcinogenic effects of ammonia or ammonia compounds are not available. Among animals, no evidence for carcinogenicity was observed in two strains of mice administered ammonium hydroxide in drinking water for two years or in a urethane-sensitive strain of mice administered ammonia in water by gavage for 4 weeks. There is some indication that ammonia contributes to the development of cancer when coadministered with DEPC (via formation of urethane) or MNNG (via stimulation of cell proliferation in the gastric mucosa). Limited genotoxicity testing of ammonia has produced mixed results. Under the proposed guidelines (U.S. EPA, 1999), the data for carcinogenicity of ammonia *are inadequate for an assessment of human carcinogenic potential.*

Derivation of quantitative estimates of cancer risk for ammonia is precluded by the absence of data indicating a carcinogenic effect for this chemical.

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