

---

## **Safety Assessment of Ammonia and Ammonium Hydroxide as Used in Cosmetics**

---

Status: Final Report  
Release Date: February 15, 2018  
Panel Date: December 4-5, 2017

The 2017 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst.

---

© Cosmetic Ingredient Review

1620 L STREET, NW, SUITE 1200 ♦ WASHINGTON, DC 20036-4702 ♦ PH 202.331.0651 ♦ FAX 202.331.0088 ♦ [CIRINFO@CIR-SAFETY.ORG](mailto:CIRINFO@CIR-SAFETY.ORG)

**ABSTRACT:** The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) reviewed the safety of Ammonia and Ammonium Hydroxide, which function as pH adjusters in cosmetic products. Ammonium Hydroxide is also reported to function as a denaturant. The Panel reviewed data relevant to the safety of these ingredients and concluded that Ammonia and Ammonium Hydroxide are safe as used in hair dyes and colors and safe in cosmetics applied directly to the skin in the present practices of use and concentration described in the safety assessment, when formulated to be non-irritating.

## **INTRODUCTION**

The safety of Ammonia and Ammonium Hydroxide in cosmetics is reviewed in this Cosmetic Ingredient Review (CIR) safety assessment. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI Dictionary), both ingredients are reported to function as pH adjusters in cosmetic products.<sup>1</sup> Additionally, Ammonia is reported to function as an external analgesic and fragrance ingredient, and Ammonium Hydroxide is reported to function as a denaturant in cosmetic products. External analgesic and fragrance are functions the CIR Expert Panel (Panel) may exclude from their purview and, therefore, they will not evaluate safety in relation to those uses.

This safety assessment includes relevant published and unpublished data for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A list of the typical search engines and websites used, sources explored, and endpoints that CIR evaluates is available on the CIR website (<http://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites>; <http://www.cir-safety.org/supplementaldoc/cir-report-format-outline>). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

An Environmental Protection Agency (EPA) toxicological review that covers gaseous Ammonia (NH<sub>3</sub>) and Ammonia dissolved in water (Ammonium Hydroxide, NH<sub>4</sub>OH) was published in 2016.<sup>2</sup> It should be noted that portions of the EPA review are adapted from the toxicological profile for Ammonia that was developed by the Agency for Toxic Substances and Disease Registry (ATSDR). This CIR safety assessment also includes toxicity data on Ammonia/Ammonium Hydroxide that have become available since the ATSDR and EPA documents were published.

In addition to the ATSDR and EPA reports on Ammonia, an expert assessment of the effects on human health and the environment posed by Ammonia, prepared by a 14-member task group, is available.<sup>3</sup> This assessment was published under the joint sponsorship of the United Nations Environment Program, the International Labor Organization, and the World Health Organization.

Furthermore, in addition to the safety test data on Ammonia and Ammonium Hydroxide that are included in this safety assessment, the Panel addressed the use of chemicals for read-across, and determined that information reported for the following chemicals is appropriate for read-across: data on "ammonium ion" (reproductive and developmental toxicity, genotoxicity, and carcinogenicity data; counter ion not identified) that are included in the ATSDR toxicological profile for Ammonia; diammonium phosphate (repeated dose (short-term) oral toxicity and reproductive and developmental toxicity data) that are included in the CIR Final Report on Phosphoric Acid and Its Salts and in the European Chemicals Agency (ECHA) registration dossier on Ammonia; and data on ammonium chloride (genotoxicity data [micronucleus test]) and ammonium sulfate (oral carcinogenicity and chronic oral toxicity data) that are included also included in the ECHA dossier (Table 1).<sup>4,5,6</sup>

## **CHEMISTRY**

### **Definition and General Characterization**

Ammonia, ammonia gas, anhydrous ammonia, and liquid ammonia are terms that are often used interchangeably to refer to the ingredient, Ammonia, in either its liquid (compressed) or gaseous state.<sup>7</sup> Ammonia dissolved in water is referred to as aqueous ammonia, ammonia solution, and the cosmetic ingredient name, Ammonium Hydroxide. In an aqueous formulation, these two ingredients will each consist of at least some of the other, dependent on the effective pH of the formulation.

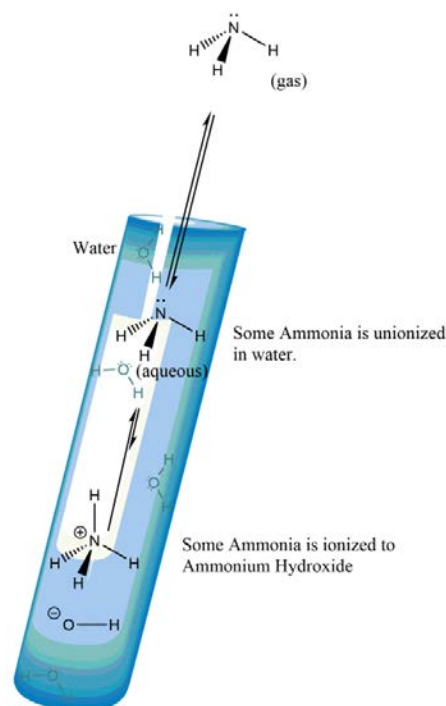


Figure 1. The gas and aqueous solution equilibria of Ammonia and Ammonium Hydroxide

Referring to Figure 1, some ammonia is actually ionized to ammonium ion, with hydroxide anion being formed in an equal amount (stoichiometrically) in pure water to produce Ammonium Hydroxide.

Ammonium Hydroxide is formed simply by the solution of Ammonia in water. Regardless of whether the ingredient is named Ammonia or Ammonium Hydroxide, if the formulation or test article contains water, both are present in equilibrium. At or near neutral pH, more than 99% is in the form of ammonium ion, and less than 1% is Ammonia. In more alkaline (i.e. higher pH) solutions, the Ammonia concentration can be significantly higher (e.g., at pH 9.25 the ratio of Ammonia to Ammonium Hydroxide is about 1:1;  $pK_b \sim 4.8$  at room temperature). Accordingly, the ratio of dissolved molecular Ammonia versus the ions of Ammonium Hydroxide is mainly dependent on the pH of the formulation. Saturation in water, at room temperature and atmospheric pressure, is approximately 34%.<sup>8</sup>

Since the functions of fragrance and external analgesic may be excluded from the purview of the CIR Expert Panel, the only function of Ammonia under review herein is pH adjuster. The term “pH” is defined as the negative  $\log_{10}$  of the concentration of hydrogen ions (protons), existing as solvated forms, including hydronium ion and higher solvates in water. Above pH 7, the concentration of hydroxyl anion ( $OH^-$ ) becomes greater than hydronium, increasing as pH becomes more alkaline. Accordingly, pH adjusters function in aqueous formulations and this safety assessment evaluates Ammonia and Ammonium Hydroxide in that context. (Ammonium Hydroxide is also reported functions as a denaturant.)

The definitions, structures, and functions in cosmetics of these ingredients are presented in Table 2.

### Chemical and Physical Properties

Ammonia is a small nitrogenous compound with a molecular weight of 17 that is a gas at standard temperature and pressure.<sup>9</sup> It is a weak base that exists in protic equilibrium with Ammonium Hydroxide, as shown in Figure 1. Solvation of ammonia in water results in an equilibrium between dissolved ammonia and ammonium, the latter being formed by the abstraction of a proton from one equivalent of water molecules to produce a stoichiometric amount of hydroxyl anions (i.e., an acid-base reaction in essentially instantaneous equilibrium when in free aqueous solution). Additional chemical properties are presented in Table 3.<sup>9,10,11,12</sup>

### Method of Manufacture

Ammonia can be formed from water gas and producer gas via the Haber-Bosch process (i.e., under high temperature and pressure, hydrogen and nitrogen are combined to produce Ammonia).<sup>8</sup>

Ammonium Hydroxide can be produced by passing Ammonia gas into water.<sup>13</sup>

### **Composition**

According to the *Food Chemicals Codex*, Ammonium Hydroxide contains not less than 27% and not more than 30% by weight  $\text{NH}_3$ .<sup>14</sup> The monograph on strong Ammonia solution in the *United States Pharmacopoeia* states that this is a solution of  $\text{NH}_3$ , containing not less than 27% and not more than 31 % (w/w)  $\text{NH}_3$ .<sup>15</sup>

### **Impurities**

According to the *Food Chemicals Codex*, the acceptance criteria for Ammonium Hydroxide include: lead (not more than 0.5 mg/kg), nonvolatile residue (not more than 0.02%), and readily oxidizable substances (pink color does not completely disappear within 10 minutes).<sup>14</sup> Similarly, according to the *United States Pharmacopoeia*, the limitations on strong Ammonia solution include: heavy metals (0.0013% limit), nonvolatile residue (not more than 5 mg of residue remains [0.05%]), and readily oxidizable substances (pink color does not completely disappear within 10 minutes).<sup>15</sup>

### **USE**

#### **Cosmetic**

The safety of Ammonia and Ammonium Hydroxide is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database.<sup>16</sup> Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.<sup>17</sup>

According to 2017 VCRP data, Ammonia is being used in 599 cosmetic products (mostly rinse-off products) and Ammonium Hydroxide is being used in 1354 cosmetic products (mostly rinse-off products) (Table 4).<sup>16</sup> Most of the uses of these 2 ingredients are in hair coloring products (582/599 (97.2%) uses for Ammonia and 1104/1354 (81.5%) uses for Ammonium Hydroxide). The results of a concentration of use survey provided by the Council in 2017 indicate that the highest maximum cosmetic use concentration of Ammonia is 4.6 % (in hair dyes and colors) and that the highest maximum cosmetic use concentration of Ammonium Hydroxide is 12.5% (in hair dyes and colors) (Table 4).<sup>17</sup> Regarding use concentrations in leave-on products, the highest maximum cosmetic use concentrations are 0.73% (Ammonia - in tonics, dressings, and other hair grooming aids) and 1.5% (Ammonium Hydroxide - in face and neck products [not spray]).

Cosmetic products containing Ammonia or Ammonium Hydroxide may be applied to the skin and hair or, incidentally, may come in contact with the eyes (e.g., at maximum use concentrations up to 0.58% (Ammonium Hydroxide in eye brow pencils) and mucous membranes (e.g., at maximum use concentrations up to 0.0012% Ammonium Hydroxide in bath soaps and detergents). Products containing Ammonia or Ammonium Hydroxide may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

The denaturant use of Ammonium Hydroxide is relevant to cosmetic use. The formula for SD alcohol 36 is: "To every 100 gallons of strong ammonia solution, N.F.: 17.5 pounds of caustic soda, liquid grade, containing 50 percent sodium hydroxide by weight; or 12.0 pounds of caustic soda, liquid grade, containing 73 percent sodium hydroxide by weight." (27CFR21.63) This denatured alcohol is permitted for use in shampoos and soap and bath preparations.

Ammonia (CAS No. 7664-41-7) and Ammonium Hydroxide (CAS No. 1336-21-6) are on the European Union's list of substances that cosmetics must not contain, except when subject to the following restriction: maximum concentration in ready for use preparation (6% (as  $\text{NH}_3$ )).<sup>18</sup> Furthermore, the following phrase appears in the wording of "conditions of use and warnings" category: above 2%: contains Ammonia.

#### **Non-Cosmetic**

Ammonia is a chemical with diverse uses, such as fertilizer and as a refrigerant.<sup>19</sup> Ammonia is also used in production of dyes, plastics, synthetic fibers, pesticides, explosives, refrigerants, and pharmaceuticals, and in the purification of water.<sup>7</sup> 27CFR21.63 also permits (in addition to the cosmetic uses above) the use of Ammonium Hydroxide as a denaturant in household cleaners.

Ammonium Hydroxide is affirmed as generally recognized as safe (GRAS) as a direct human food ingredient. [21CFR184.1139] Ammonium Hydroxide must meet the specifications of the *Food Chemicals Codex* (see Impurities section), and, in accordance with these specifications, the ingredient is used in food with no limitation other than current good manufacturing practice. Concerning animals, anhydrous Ammonia is a food additive permitted in feed and drinking water, and it is used or intended for use as a source of non-protein nitrogen in cattle feed. [21CFR573.180]

In Australia, Ammonia and Ammonium Hydroxide are listed in the *Poisons Standard*, the standard for the uniform scheduling of medicines and poisons (SUSMP) in schedules 5 and 6.<sup>20</sup> Under schedule 5, Ammonia and Ammonium Hydroxide are permitted in preparations containing  $\leq 5\%$  Ammonia, with the following exceptions: in preparations for human internal therapeutic use; in preparations for inhalation when absorbed in an inert solid material; or in preparations containing  $\leq 0.5\%$  free Ammonia. Schedule 5 chemicals are defined as substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label; schedule 5 chemicals are labeled with "Caution". Schedule 6 chemicals are classified as poisons with a moderate potential for harm.

Ammonia, as an intravenously-injected prescription drug, is included on the list of FDA-approved drug products.<sup>21</sup> Ammonia solution has been classified as an over-the-counter (OTC) drug active ingredient as a skin protectant and external analgesic, and the same is true for Ammonium Hydroxide as a skin protectant. However, FDA has determined that there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses.<sup>22</sup>

### **TOXICOKINETIC STUDIES**

***Because of the equilibrium nature of these two ingredients, the studies that follow will simply recite "Ammonia" for most cases, regardless of whether Ammonia or Ammonium Hydroxide was reported.***

#### **Absorption, Distribution, Metabolism, and Excretion**

Ammonia is the principal by-product of amino acid metabolism, and the liver is indicated as the central organ of Ammonia metabolism.<sup>23</sup> It is generated, in vivo, from the breakdown of nitrogenous substances in the gut and from the use of glutamine as a metabolic fuel in the small intestine, and is taken up by the liver where it is detoxified by conversion to urea and, to a lesser extent, glutamine.<sup>24,25</sup> The main source of in vivo Ammonia generation occurs in the intestines, from lysis of blood-borne urea and also from protein digestion/deamination by urease-positive bacteria and microbial deaminase.<sup>26,27</sup> A large amount of metabolically-generated Ammonia is absorbed into the blood and, via the portal vein, is transported to the liver where it is detoxified.<sup>26,28,29</sup> The normal concentration of Ammonia in the portal blood varies from 300 to 600  $\mu\text{M}$ . But, in the blood leaving the liver the concentration is reduced to 20–60  $\mu\text{M}$ . The liver occupies a central position in the regulation of Ammonia levels in the organism.<sup>30,31</sup> According to another source, the normal range for blood serum levels is 10–40  $\mu\text{mol/L}$ .<sup>32</sup>

The substrates from which Ammonia may be formed in the gut comprise derivatives of ingested nitrogenous material, epithelial and bacterial debris, and compounds secreted from the circulation to the mucosal cells and lumen (e.g., certain peptides, amino acids, and smaller diffusible substances such as urea).<sup>33</sup> Both the gut and kidneys generate substantial amounts of Ammonia from the deamidation of glutamine.<sup>23</sup> The glutamine-glutamate cycle in the body works in conjunction with the glucose-alanine cycle to shuttle Ammonia from peripheral to visceral organs.

Renal regulation of acid-base balance involves the formation and excretion of Ammonia to buffer hydrogen ions that are excreted in the urine. Approximately two-thirds of urinary Ammonia is derived from the amide nitrogen of glutamine, a reaction that is catalyzed by the glutaminase enzyme in renal tubular cells.<sup>23</sup>

The first step in the degradation of most amino acids is the removal of an  $\alpha$ -amino residue, and an amino residue is transferred to  $\alpha$ -ketoglutaric acid to produce glutamate.<sup>34</sup> Glutamate dehydrogenase converts glutamate to  $\alpha$ -ketoglutarate and Ammonia. To prevent a toxic buildup of Ammonia, it is converted to glutamine and alanine in a number of tissues for transportation to the liver. Ammonia is then converted to urea via the urea cycle in the liver, and urea is excreted in the urine. The urea cycle, a cycle of biochemical reactions that produces urea from Ammonia, is the major pathway for Ammonia detoxification in terrestrial mammals.<sup>35</sup> In the liver, the urea cycle is essential to the conversion of excess nitrogen from Ammonia and aspartate into urea.<sup>36</sup> When the supply of Ammonia in mammals exceeds the capacity for its detoxification, the excretion of orotic acid in the urine increases.<sup>35</sup> Orotic acid (from the urea cycle) is an intermediate product in the biosynthesis of pyrimidines and purines.

There is evidence that Ammonia can cross the blood-brain barrier (BBB), mostly through ion transporters rather than by passive diffusion of gaseous Ammonia.<sup>26,37</sup>

## **Animal**

### **Inhalation**

Brain glutamine levels have been shown to increase in rats that inhaled 25 or 300 ppm Ammonia vapor for 6 hours/day for 5 days, which is likely a result of Ammonia metabolism by the astrocytic glutamate-glutamine cycle.<sup>38,39</sup>

Continuous exposure of rats for 24 h to concentrations up to 32 ppm Ammonia resulted in significant increase in blood Ammonia levels.<sup>40</sup> Exposures to 310 - 1157 ppm led to significantly increased blood concentrations of Ammonia within 8 h of exposure initiation, but blood Ammonia returned to pre-exposure values within 12 hours of continuous exposure and did not change over the remainder of the 24-hour exposure period.

### **Parenteral**

Following the administration of [<sup>13</sup>N]Ammonia to rats (via either the carotid artery or cerebrospinal fluid), most metabolized labelled nitrogen was in glutamine (amide), and little was in glutamate (plus aspartate).<sup>41</sup>

## **TOXICOLOGICAL STUDIES**

### **Acute Toxicity Studies**

#### **Dermal**

Acute dermal toxicity studies on Ammonia were not found in the published literature, nor were these data submitted.

Acute oral toxicity studies summarized below are presented in Table 5, and acute inhalation studies are summarized below and presented in Table 6.

#### **Oral**

In a study in which groups of 6 rats were dosed orally with Ammonium Hydroxide at concentrations ranging from 0.03% to 1%, the minimum concentration at which gastric lesions were observed was 0.03%; the ulcer index increased in a concentration-dependent manner.<sup>42</sup> An acute oral LD<sub>50</sub> of 350 mg/kg for Ammonia in rats (groups of 10) has been reported,<sup>6,43,44</sup> and the oral administration of 1 % or 3% (w/w as Ammonium Hydroxide) to groups of 4 to 8 rats by gavage has produced severe hemorrhagic lesions of the stomach.<sup>45</sup>

#### **Inhalation**

In a 10-minute exposure study involving mice exposed to Ammonia at concentrations ranging from 8723 to 12,870 ppm, an LC<sub>50</sub> of 10,096 ppm was reported.<sup>24,46</sup> An LC<sub>50</sub> of 10,150 ppm was reported in another 10-minute exposure study in which mice were exposed to Ammonia at concentrations ranging from 8770 to 12,940 ppm.<sup>47,46,48</sup> In mice exposed to Ammonia (100-800 ppm) for 30 minutes, an RD<sub>50</sub> (exposure concentration that produced a 50% reduction in respiratory rate) of 303 ppm was reported.<sup>24,49,48</sup> The following effects were observed in mice that were exposed to Ammonia at a concentration of 21,400 ppm for 30 minutes: eye irritation, dyspnea, histopathological changes in the lungs (alveolar disruption and loss of septal continuity), coma, and death.<sup>24,50</sup> The following results were observed in mice that were exposed to Ammonia for 1 h at various concentrations: nasal/eye irritation, liver congestion, and LC<sub>50</sub> = 4837 ppm (concentration range: 3600 to 5720 ppm);<sup>24,51,48</sup> mild to moderate pneumonitis, hepatic lesions, and LC<sub>50</sub> = 4230 ppm (concentration range: 1190 to 4860 ppm);<sup>24,52,48</sup> eye irritation and alveolar disruption (at 4840 ppm);<sup>24,53</sup> and liver necrosis (at 3440 ppm).<sup>52</sup>

In acute inhalation toxicity studies involving rats, LC<sub>50</sub>s ranging from 7068 ppm<sup>54</sup> to 40,300 ppm<sup>55</sup> and RD<sub>50</sub>s of 1396 ppm and 1299 ppm<sup>24,56</sup> have been reported. Exposure durations included 10 minutes (14,170 to 54,289 ppm; LC<sub>50</sub> = 40,300 ppm),<sup>55</sup> 20 minutes (14,170 to 54,289 ppm; LC<sub>50</sub> = 28,595 ppm),<sup>55</sup> 40 minutes (14,170 to 54,289 ppm; LC<sub>50</sub> = 20,300 ppm),<sup>55</sup> 1 h (14,170 to 54,289 ppm; LC<sub>50</sub> = 16,600 ppm),<sup>55</sup> 1 h (3028-14,044 ppm; LC<sub>50</sub> = 17,633 ppm),<sup>54</sup> and 4 h (3028-14,044 ppm; LC<sub>50</sub> = 7068 ppm).<sup>54</sup> Signs of upper respiratory tract irritation were observed within the following concentration ranges: 14,170 to 54,289 ppm;<sup>55</sup> 9000 to 35,000 ppm (LC<sub>50</sub> = 23,672 ppm);<sup>57</sup> 9,000 to 23,000 ppm;<sup>58</sup> 3028 to 14,044 ppm;<sup>54</sup> and 1436, 4307, and 6814 ppm.<sup>59</sup> No effects were observed at a concentration of 144 ppm.<sup>60</sup> Increased production of respiratory tract fluid was observed in rabbits after a 1-h exposure to Ammonia at the following concentrations: 5200 to 12,800 ppm;<sup>24</sup> average concentration of 10,360 ppm;<sup>24</sup> and at 3.5 and 8.7 ppm.<sup>61</sup> A significant decrease in respiratory rate was observed in rabbits exposed to Ammonia (50 and 100 ppm) for 2.5 to 3 h.<sup>62</sup> Congestion of the respiratory tract/lungs was reported in cats exposed to 1000 ppm Ammonia for 10 minutes<sup>63,64</sup> and, for 1 h, to 5200 ppm to 12,800 ppm Ammonia,<sup>47,65</sup> an average concentration of 10,360 ppm,<sup>47,65</sup> and 3.5 and 8.7 ppm.<sup>66</sup> Gross pathological findings in the

respiratory tract after the 10-minute exposure included varying degrees of congestion, hemorrhage, edema, interstitial emphysema, and lung collapse.

It has been noted that acute exposure data have demonstrated that injury to respiratory tissues is primarily due to Ammonia's alkaline (i.e., caustic) properties, resulting from the formation of hydroxide when Ammonia reacts with water.<sup>2</sup> Furthermore, Ammonia readily dissolves in the moisture on mucous membranes, forming Ammonium Hydroxide, which causes liquefactive necrosis of the tissues.

### **Short-Term Toxicity Studies**

#### **Animal**

##### **Dermal**

Short-term dermal toxicity data on Ammonium Hydroxide or Ammonia were not found in the published literature, nor were these data submitted.

Short-term oral and inhalation toxicity studies are summarized below and presented in Table 7.

##### **Oral**

Mucosal atrophy in the stomach antrum and enlargement of the proliferative zone in the mucosa of the stomach antrum and body were observed in rats that received 0.01% Ammonium Hydroxide (in drinking water) for 8 weeks.<sup>67</sup> A no-observed-adverse effect-level (NOAEL) of 250 mg/kg/day for general toxicity and a lowest-observed-adverse effect-level (LOAEL) of 750 mg/kg/day for general toxicity were reported for diammonium phosphate (identified as an appropriate read across material for short-term oral toxicity and included herein to support the overall weight of evidence) in rats dosed orally (by gavage) for 5 weeks.<sup>6,48</sup>

##### **Inhalation**

Rhinitis and pathological lesions with metaplasia and necrosis were observed in mice exposed to Ammonia for 14 days at a concentration of 711 ppm, but not at concentrations of 78 and 271 ppm.<sup>24,68</sup> Histopathologic findings (nasal cavity) for mice exposed to Ammonia (303 ppm) repeatedly for 5 days included slight squamous metaplasia, ulceration, necrosis, and moderate inflammatory changes.<sup>24,69</sup> Lung congestion and edema were observed in mice exposed to Ammonia (20 ppm) for 42 days.<sup>2,70</sup> A low incidence of carcinoma of the nasal mucosa was observed in mice exposed to Ammonia (12% solution) for 8 weeks, and these results are summarized in more detail in the Carcinogenicity section.<sup>2,71</sup> Rats were exposed repeatedly to Ammonia at concentrations ranging from 10 or 150 ppm (for 75 days)<sup>2,48,72</sup> to 1306 ppm (for 42 days (5 days/week and 8 h/day)).<sup>73</sup> The higher concentration was tolerated for 42 days in rats, and increased thickness of the nasal epithelium was observed at 150 ppm, but not 10 ppm. Nasal irritation and inflammation of the upper respiratory tract were observed in rats exposed to 500 ppm Ammonia for 3 weeks; reactions had cleared by week 8.<sup>48,74</sup>

When rats, rabbits, guinea pigs, monkeys, and dogs were exposed to Ammonia at a concentration of ~ 223 ppm or ~ 1105 ppm for 6 weeks (5 days/week and 8 h/day), the following effects were observed: focal pneumonitis in 1 of 3 monkeys at 223 ppm; nonspecific lung inflammation in guinea pigs and rats, but not other species at 1105 ppm; and mild to moderate dyspnea in rabbits and dogs during week 1 only at 1105 ppm.<sup>2,40,73</sup> Upper respiratory effects (e.g., dyspnea and nasal lesions, irritation, and inflammation) were observed over most of the range of concentrations tested (145 ppm (5-week exposure)<sup>2,75</sup> to 1306 ppm (42-day exposure (5 days/week and 8 h/day))<sup>73</sup> in short-term inhalation toxicity studies on Ammonia involving mice, rats, guinea pigs, pigs, rabbits or dogs. At lower Ammonia concentrations, there were no treatment-related effects in rats (at 50 or 90 ppm (continuous exposure for 50 days))<sup>48,76</sup> and there was no increase in the incidence of respiratory diseases in pigs exposed to Ammonia (37 ppm or ~ 14.2 ppm, inhalable dust exposure) for 5 weeks.<sup>2,77</sup> In other studies, nearly 64% lethality was reported for rats exposed to Ammonia (653 ppm) for 25 days (continuous exposure)<sup>9</sup> and 50 of 51 rats exposed to Ammonia (650 ppm) were dead by day 65 of continuous exposure.<sup>46,73</sup> In a study in which pigs were exposed to Ammonia (5 to 100 ppm) for 6 days, nasal irritation was observed at concentrations down to 25 ppm.<sup>78</sup>

### **Subchronic Toxicity Studies**

Neither subchronic dermal nor oral toxicity data on Ammonium Hydroxide or Ammonia were found in the published literature, nor were these data submitted.

## **Inhalation**

Subchronic inhalation toxicity studies on Ammonia and Ammonium Hydroxide are summarized below and presented in Table 7.

Fatty changes of liver plate cells were seen in rats following continuous exposure to Ammonia (642 ppm) for 90 days.<sup>9</sup> The following results were reported for guinea pigs exposed to ~170 ppm Ammonia for 18 weeks: mild congestion of the liver, spleen, and kidneys; degenerative changes in the adrenal glands; hemosiderosis in the spleen; and cloudy swelling in proximal kidney tubules.<sup>47,81</sup> Damaged tracheal mucosae were observed in rats exposed repeatedly to Ammonia (100 ppm) for 12 weeks.<sup>47,82</sup> Mild leukocytosis was noted in rats after exposure to 143 ppm, but not 43 ppm, Ammonia repeatedly (25- or 60-minute exposures every 48 h) for 3 months.<sup>48</sup>

A low incidence of mortalities (13 of 515 rats and 4 of 15 guinea pigs) was observed in rats and guinea pigs exposed continuously to 671 ppm Ammonia for 90 days. However, there were no mortalities or treatment-related histopathological findings in rats, guinea pigs, rabbits, monkeys, or dogs exposed continuously to ~57.43 ppm Ammonium Hydroxide for 114 days.<sup>48,73</sup>

## **Chronic Toxicity Studies**

### **Animal**

#### **Dermal**

Chronic dermal toxicity data on Ammonium Hydroxide/Ammonia were not found in the published literature, nor were these data submitted.

#### **Oral**

Enlarged adrenal glands were observed in rabbits that received 124 mg ammonium/kg/day as (as Ammonium Hydroxide) by gavage in water for 17 months.<sup>83</sup>

#### ***Ammonium Sulfate (read-across for Ammonia)***

Limited chronic oral toxicity data were available for Ammonia. However, ammonium sulfate was identified as an appropriate read-across surrogate. The chronic oral toxicity of ammonium sulfate was evaluated using groups of 10 Fischer 344/DuCrj rats (males and females). Ammonium sulfate was administered in the diet daily at concentrations of 0%, 0.1%, 0.6%, and 3% for 52 weeks. None of the animals died, and there were no macroscopic findings. There was a significant increase in kidney and/or liver weights in males and females of the 3% dietary group, but there were no effects on survival rate, body weights, or hematological, serum biochemical, or histopathological parameters at any concentration. Several non-neoplastic lesions, such as bile duct proliferation in the liver and focal myocarditis in the heart were observed in the 3% dietary group, but also in animals of the control group, and the difference in results was not statistically significant when the 2 groups were compared. The NOAEL for ammonium sulfate was estimated to be 0.6% in both sexes, which is equivalent to 256 and 284 mg/kg/day in males and females, respectively.<sup>6,84</sup> Neoplastic lesions (classified as unrelated to ammonium sulfate in the diet) reported in a carcinogenicity study in the same report are included in Table 9.

## **DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES**

Developmental/reproductive toxicity studies are summarized below and presented in Table 8.

A relationship between the duration of exposure and the incidence of exencephaly (concentration-related increase) was observed in an *in vitro* study in which mouse embryos were cultured with Ammonia (38 to 300  $\mu\text{mol/l}$ ) for up to 93 h.<sup>89</sup> In a developmental toxicity study involving pregnant rats exposed to Ammonia in the diet (4293 mg/kg/day; as the ammonium ion [read-across for Ammonia]) from gestation day 1 through day 21 of lactation, body weights of offspring were reduced by 25% (males) and 16% (females).<sup>9,90</sup> Neither reproductive nor developmental toxicity was reported in a study in which female pigs were exposed (inhalation exposure) to ~7 ppm or ~35 ppm Ammonia from 6 weeks prior to breeding until day 30 of gestation.<sup>91</sup> In an oral reproductive and developmental toxicity study on diammonium phosphate (identified as an appropriate read across material for developmental and reproductive toxicity and included herein to support the overall weight of evidence) involving rats in which embryos were treated *in vitro* and then implanted, an NOAEL of 1500 mg/kg/day (highest dose tested) was reported.<sup>6,44</sup> The only histological findings relating to maternal toxicity in this study were the inflammatory/degenerative changes in all treatment groups (diammonium phosphate at 250, 750, and 1500 mg/kg/day),



which were considered likely to have been the result of an irritant effect. No statistically significant differences in the number of live fetuses, fetal length, or fetus-to-corpus luteum ratio were observed after gilts were exposed (inhalation) to Ammonia (7 or 35 ppm) from 6 weeks prior to breeding until day 30 of gestation.<sup>91</sup>

## **GENOTOXICITY STUDIES**

### **In Vitro**

Ammonia was non-genotoxic when tested at concentrations up to 25,000 ppm (with and without metabolic activation) in the following bacterial strains: *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA1538, and *Escherichia coli* strain WP2 uvr A.<sup>6,48,44</sup>

Ammonia was non-genotoxic to *E. coli* strain Sd-4-73 in an in vitro assay without metabolic activation.<sup>44</sup>

### **In Vivo**

Blood samples from 22 workers who had been exposed to Ammonia (concentrations unknown) in a fertilizer factory were compared with samples obtained from 42 unexposed controls. Results (compared to controls) were as follows: increased frequency of chromosomal aberrations (CAs), sister chromatid exchanges (SCEs), and mitotic index, with increasing duration of exposure. Smokers had higher SCE and CA values than non-smokers and alcoholics had more CAs and SCEs than non-alcoholics.<sup>92</sup>

### ***Ammonia and Ammonium Chloride (read-across for Ammonia)***

An increased frequency of micronuclei (compared to controls) was observed in Swiss albino mice that received single intraperitoneal (i.p.) doses of Ammonia (12, 25, or 50 mg/kg). The maximum number of micronucleated polychromatic erythrocytes was associated with mice that received the highest dose (50 mg/kg), and there was clear correlation of dose-yield effects. In another micronucleus test, groups of 10 (5 males, 5 females) ddY mice received single i.p. doses of 62.5, 125, 250 and 500 mg/kg ammonium chloride (identified as an appropriate read across material for in vivo genotoxicity and included to support the overall weight of evidence) or i.p. doses of 31.3, 62.5, 125, and 250 mg/kg ammonium chloride (4 injections within 24 h). Femoral bone marrow cells were examined for polychromatic erythrocytes, and there was no evidence of genotoxicity at the doses of ammonium chloride that were administered.<sup>6,92</sup>

## **CARCINOGENICITY STUDIES**

Carcinogenicity and tumor promotion studies are summarized below and presented in Table 9.

There was no evidence of a tumorigenic effect in Kid: CFLP mice treated by gavage with ammonia dissolved in water alone at a dose of 42 mg Ammonia /kg/day for 4 weeks or with diethyl pyrocarbonate alone, but 9 of 16 mice treated with a combination of ammonium ion and diethyl pyrocarbonate developed lung tumors.<sup>93</sup> Following the oral dosing of mice (Swiss and C3H) with Ammonia (in drinking water, 193 mg/kg/day) for 2 years, there was no evidence of carcinogenicity and no effect on the spontaneous development of adenocarcinoma of the breast (associated with C3H mouse strain).<sup>44,48,94</sup> Ammonium sulfate was classified as non-carcinogenic in rats in a study involving dietary concentrations up to 3% daily for 104 weeks.<sup>84</sup> Neoplastic lesions were observed in this study, but were deemed not treatment-related because of the spontaneous occurrence of these lesions in the rat strain (F344/DuCrj) that was tested. Malignant pheochromocytoma of the adrenal gland and pituitary adenomas were observed in F344/DuCrj rats fed 3% ammonium sulfate, but not 0.1% or 0.6%, in the diet for 52 weeks.<sup>6</sup> After pregnant HSL mice were exposed (by gavage) to ammonium ion + diethyl pyrocarbonate during pregnancy and lactation, there was no evidence of lung tumors in mice treated during pregnancy or in the suckling mice of dams treated during lactation.<sup>95</sup> When 10 mice were exposed (vapor inhalation) to 12% Ammonia solution repeatedly for 8 weeks, carcinoma of the nasal mucosa was observed in 2 mice.<sup>71</sup>

When 10 mice were exposed (vapor inhalation) to 12% Ammonia solution repeatedly for 8 weeks, carcinoma of the nasal mucosa was observed in 2 mice.<sup>71</sup>

### **Tumor Promotion**

A statistically significant increase in the incidence of gastric cancer (70%) was observed in rats dosed orally with the initiator *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and 0.01% Ammonia, when compared to dosing with MNNG alone.<sup>96</sup> In another study, the size, depth, and metastasis of MNNG-initiated tumors was enhanced in rats dosed orally with Ammonia (~42 mg/kg/day).<sup>97</sup>

## **OTHER RELEVANT STUDIES**

### **Neurotoxicity**

Ammonia is most toxic in the brain, and chronic hyperammonemia (metabolic disturbance) may lead to brain damage, especially in children.<sup>23</sup> During normal body function, approximately 10% of arterial Ammonia is extracted by the brain. Neurotoxicity is observed only when circulating levels of Ammonia are elevated. It has been reported that hyperammonemia is associated with neuronal cell loss and cerebral atrophy that lead to mental retardation and cerebral palsy in pediatric patients.<sup>98</sup> These toxic effects are specific to the developing brain, as neuronal damage is not observed in the brain of adult patients with hyperammonemia due to liver failure.

According to another source, many neurologic disorders are related to congenital or acquired hyperammonemia. Evidence obtained with the use of experimental hyperammonemia models suggests that acute neurotoxic effects of Ammonia are mediated by over activation of ionotropic glutamate receptors, mainly the *N*-methyl-D-aspartate (NMDA) receptors, and, to a lesser degree, the kainic acid (KA)/ $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors.<sup>99</sup> Results from other studies suggest that glutamine is also a mediator of Ammonia neurotoxicity.<sup>100,101</sup>

Toxic levels of Ammonia and alterations in pH, electrolyte disturbances, and membrane potential depolarization are thought to lead to neurological dysfunction, primarily by causing cellular swelling accompanied by brain edema and metabolic dysfunction.<sup>26,102</sup> Studies have suggested that Ammonia is likely to be particularly toxic to astrocytes, as they are the only cells that possess the enzyme glutamine synthetase (which is responsible for detoxifying Ammonia in the brain through condensation with glutamate).<sup>103,104</sup>

In *in vitro* studies, it has been demonstrated that acute intoxication with large doses of Ammonia leads to excessive activation of NMDA receptors.<sup>105,106,107,108</sup> Furthermore, excessive activation of NMDA receptors leads to neuronal degeneration and death, and is responsible for most of the neuronal damage that is found in brain ischemia.<sup>105</sup>

### **Brain Pathology**

The excessive formation of Ammonia within the brains of Alzheimer's disease patients and its release into the periphery has been demonstrated.<sup>109,110</sup> Furthermore, a higher expression of AMP-deaminase in the brains of Alzheimer's disease patients has been observed, and this finding indicates the existence of a pathologically elevated source of Ammonia within the brain of Alzheimer's disease patients.<sup>109,111</sup>

### **Cytotoxicity**

Lymphocytes separated from peripheral bovine (Holstein-Friesian cows) blood were incubated for 2 h in control medium and test medium with various concentrations of Ammonia (w/v as Ammonium Hydroxide; 0.01 mg/dl, 0.1 mg/dl, 1 mg/dl, and 10 mg/dl).<sup>112</sup> Viability of the lymphocytes, measured by trypan blue exclusion test, was significantly reduced after 2 h of incubation. At a concentration of 0.01 mg/ml, lymphocyte viability was significantly reduced after 24 h and 48 h of incubation. In another experiment, in which lymphocytes were preincubated with Ammonia (w/v as Ammonium Hydroxide; 10 mg/dl) and then washed and resuspended in the fresh medium with Ammonia, the number of viable cells was reduced to 51%  $\pm$  8 at 24 h, 40%  $\pm$  7 at 48 h, and to 39%  $\pm$  6 at 72 h of incubation.

### **Effect on Mitosis**

The ability of Ammonia to affect the mitogenic response of bovine lymphocytes to phytohemagglutinin (PHA) or concanavalin A (Con A) was examined.<sup>112</sup> Lymphocytes from 10 Holstein-Friesian cows were incubated with various concentrations of PHA and Ammonia. Lymphocytes from 6 cows were incubated with Con A and Ammonia. Mitogenic reactivity was measured by the incorporation of methyl-<sup>3</sup>H-thymidine into the DNA of lymphocytes. Ammonia at concentrations of 0.01 mg/dl (w/v as Ammonium Hydroxide) significantly ( $P < 0.01$ ) suppressed PHA (optimal dose = 0.5  $\mu$ g/ml) stimulation of lymphocytes from only 1 animal. Other concentrations of Ammonia, at 0.1 mg/dl, 1 mg/dl, and 10 mg/dl (w/v as Ammonium Hydroxide), significantly ( $P < 0.01$ ) reduced the response to PHA of lymphocytes from 5 cows, 9 cows, and from all animals, respectively. These concentrations significantly reduced Con A (optimal dose = 0.5  $\mu$ g/ml) stimulation of lymphocytes from 1 animal, 5 animals, and all animals, respectively. A significant suppression ( $P < 0.01$ ) of blastogenesis of lymphocytes from 1 cow by 0.01 mg/dl, 6 by 0.1 mg/dl, 14 by 1.0 mg/dl, and from 16 cows by 10.0 mg/dl was observed. The mitogenic response of lymphocytes was reduced when lymphocytes were preincubated with Ammonia for a duration as short as 1 h.

### **Generation of Free Radicals**

Elevated concentrations of Ammonia have been shown to generate free radicals in rats and rat cell cultures (liver and brain),<sup>113,114</sup> leading to excessive production of nitric oxide (NO) by stimulating the citrulline-NO cycle.<sup>115</sup>

### Immunological Effects

A delayed-type hypersensitivity test was used to evaluate cell-mediated immunity in groups of 8 Hartley guinea pigs.<sup>116</sup> The animals were vaccinated with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) and exposed to Ammonia (< 15 ppm, 50 ppm, or 90 ppm (6.75 µg of Ammonia per deciliter of air)) for 3 weeks. Exposure to Ammonia was followed by intradermal challenge with a purified protein derivative of tuberculin. Dermal lesion size was reduced in animals that were exposed to Ammonia at a concentration of 90 ppm (mean diameter of dermal lesion = 8.7 mm, statistically significantly different from control ( $p < 0.05$ )). Results were not statistically significant in the 2 other exposure groups. Also, blood and bronchial lymphocytes were harvested from guinea pigs exposed to Ammonia, and the cells were then stimulated with the mitogens PHA or Con A. Reduced T-cell proliferation was observed; however, bactericidal activity in alveolar macrophages isolated was not affected. In an *in vitro* experiment in which lymphocytes and macrophages were isolated from unexposed Hartley guinea pigs and then treated with Ammonia, reduced proliferation and bactericidal capacity were observed only at concentrations that reduced viability. Specifically, the response of blood lymphocytes to Ammonia at 1.0 mg/dl and 10 mg/dl, but not  $\leq 0.1$  mg/dl, significantly reduced ( $p < 0.01$ ) the response to phytohemagglutinin stimulation. The exposure of alveolar macrophages to Ammonia at 1 mg/dl and 5 mg/dl, but not 0.01 mg/dl or 0.1 mg/dl, caused significant inhibition ( $p < 0.01$ ) of bactericidal activity. These results were indicative of nonspecific effects of Ammonia-induced immunosuppression. The authors noted that the data in this study indicate that T cells may be the target of Ammonia exposure, in that specific macrophage effects were not observed.

### Neurological Effects

Acute inhalation exposure to low levels of Ammonia (100 or 300 ppm) for 6 h continuously has been shown to depress free-access wheel running behavior in rodents.<sup>117</sup>

## DERMAL IRRITATION AND SENSITIZATION STUDIES

### Irritation

Dermal irritation studies are summarized in Table 10.

An undiluted Ammonia solution (as 30% Ammonium Hydroxide) was classified as a corrosive material after topical application to the stratum corneum surface in reconstructed human skin cultures *in vitro*.<sup>118</sup> At histologic examination of the cultures, epidermal necrosis was observed. The minimum concentration of Ammonia that caused an inflammatory reaction when applied (single application) to the skin of rats and mice (6 per species) was > 25% (rats) and = 25% (mice).<sup>119</sup> In a skin irritation study in which groups of 4 rats, guinea pigs, and mice were injected intradermally with Ammonia (0.01 ml), the minimum concentration that caused a positive reaction was 0.05% in rats, mice, and guinea pigs.<sup>119</sup> Ammonia (20% as Ammonium Hydroxide) was corrosive to the skin of rabbits.<sup>20,44</sup> In another study involving rabbits, 12% aqueous Ammonia was corrosive to the skin, whereas 10% was not.<sup>6</sup>

In clinical testing, the application of a saturated aqueous solution of Ammonia to the skin of 16 subjects resulted in blister formation and skin irritation.<sup>120</sup> In a study involving 110 subjects, Ammonia (1:1 aqueous solution) was applied to the skin and minimal blistering time (MBT) served as an indicator of cutaneous irritability.<sup>121</sup> The inflammatory reaction observed was considered slight, and MBT ranged from 3 to 57 minutes. Results from another study in which 50% Ammonium Hydroxide solution (0.5 ml of freshly prepared 1:1 aqueous solution of Ammonium Hydroxide) was applied to the skin indicated that the time required to produce a full blister was greatly prolonged in the aged, when compared to young adults.<sup>122</sup>

### Sensitization

Skin sensitization data on Ammonia were not found in the published literature, nor were these data submitted.

## OCULAR IRRITATION STUDIES

Ocular irritation studies are summarized in Table 11.

Ammonia was classified as a severe ocular irritant in the *in vitro* <sup>51</sup>Cr-release assay involving human corneal endothelial cell cultures.<sup>123</sup> In rabbits, Ammonia (as Ammonium Hydroxide) at 1 mg was classified as an ocular irritant.<sup>44</sup> At a concentration of 28.5%, Ammonia induced corneal opacity in rabbits.<sup>9,124</sup> In a study involving groups of 6 rabbits, Ammonium Hydroxide caused conjunctivitis at concentrations of 1% to 10%, but not 0.3%; the 10% concentration also caused corneal opacities within 1 h of instillation.<sup>125</sup> Conjunctivitis and corneal damage were also observed in a study involving 3 rabbits, whereby 3% Ammonia, 100 µl was instilled into the eyes.<sup>126</sup> <sup>9,44,123,124,125,126</sup>

In a study involving rats, there was no evidence of ocular irritation following exposure to Ammonia at vapor concentrations ranging from 15 to 1157 ppm.<sup>24,40</sup>

### **MUCOUS MEMBRANE IRRITATION STUDIES**

The stomachs of male Sprague-Dawley rats were exposed (mounted in an ex vivo gastric chamber) to 2 ml of Ammonia (15-60 mmol/l, in saline) for 15 minutes (for microscopic study) or for 60 minutes (for macroscopic study), and exposure was followed by examination for mucosal lesions. Microscopic damage to the gastric mucosa was observed.<sup>127</sup>

### **CLINICAL STUDIES**

#### **Case Reports**

A 68-yr-old male patient, employed for 18 years, was exposed frequently to anhydrous Ammonia leaks from a microfilm processor camera while on the job. He was diagnosed with interstitial lung disease and severe restrictive lung disease due to Ammonia inhalation. Marked diffuse interstitial fibrosis throughout the lung was observed.<sup>128</sup>

A male custodian had used Ammonia (28% Ammonium Hydroxide solution; which he dilutes in water) to clean office floors daily for 19 years.<sup>129</sup> He experienced regular episodes of upper airway irritation, coughing, and eye irritation when mixing the chemical in water. An evaluation of the patient revealed a negative rheumatoid factor and positive antinuclear antibody at a 1:320 dilution. The gallium lung scan was normal, but pulmonary function testing indicated a moderate restrictive defect and a formal exercise study indicated ventilator restriction upon attainment of maximum oxygen consumption. The results of a transbronchial lung biopsy with fiberoptic bronchoscopy revealed interstitial fibrosis with chronic inflammation. Granulomata were not present and cultures for tuberculosis and fungal infection were negative. A decrease in the diameter of the hypopharynx, secondary to hypertrophy of the soft tissues in the hypopharynx, was also observed. The opacification of the optic lens capsule, bronchiectasis, and fibrous obliteration of the small airways observed were described as chronic lesions that follow acute exposure to Ammonia.

#### **Other Clinical Reports**

Clinical reports relating to inhalation exposure are summarized in Table 12.

In various clinical reports, individuals were exposed to Ammonia at concentrations ranging from 25 ppm to 700 ppm. The periods of exposure ranged from 5 minutes to 6 weeks (5 days per week [2-6 h/day]). Nose, throat, and eye irritation were observed.<sup>9,47,53,79,130,131,132,133</sup>

### **EPIDEMIOLOGICAL STUDIES**

#### **Non-Cancer Endpoints**

A retrospective study was performed to assess the association between petrochemical exposure and spontaneous abortion. Study participants included 2853 non-smoking women who had been pregnant at least once, 96 of whom had been exposed to Ammonia (actual exposure levels unknown). Exposure during the pre-conception period and the first trimester of pregnancy was calculated based on information on perceived Ammonia exposure. Exposure during the first, second, and third trimesters was recorded separately for each pregnancy. Data analyses did not indicate any effect on spontaneous abortion (Odds ratio: 1.2; 95% confidence interval (CI): 0.5-2.60).<sup>6</sup>

### **OCCUPATIONAL EXPOSURE**

#### **Airway Inflammation**

Airway inflammation due to Ammonia exposure was evaluated in female Palestinian hairdressers in Hebron who had worked in this profession for  $12 \pm 7$  years.<sup>134</sup> Study participants included 33 non-smoking hairdressers (from 13 salons total) and 35 non-smoking control subjects. The hairdressers worked in relatively small-sized salons with scarce ventilation, lacked respiratory protective equipment, and were exposed to Ammonia concentrations ranging from 3 to 61 mg/m<sup>3</sup> (geometric mean = 7.3 mg/m<sup>3</sup>; geometric standard deviation = 3). The longest and shortest durations of Ammonia measurement in a salon were 45 minutes and 305 minutes, respectively. Peak exposure to Ammonia was evaluated by obtaining the number and duration of peaks above 10.5 mg/m<sup>3</sup> for each measurement session in a salon. The percentage of total sampling time when Ammonia levels exceeded 10.5 mg/m<sup>3</sup> was very high in 3 salons (percentages of 92%, 99.6%, and

75%, respectively). Both groups completed a questionnaire relating to respiratory symptoms during the past 12 months, and lung function and exhaled nitric oxide tests were performed. Blood and sputum samples were collected from all participants. When compared to unexposed controls, hairdressers were found to have a higher level of sputum neutrophil count (i.e., sign of neutrophilic airway inflammation; absolute numbers of neutrophils per mg sputum: 376 (for hairdressers) versus 182 (for controls)). The hairdressers also had significantly elevated exhaled nitric oxide levels and blood C reactive protein levels when compared to control subjects (controlled for age and body mass index).

### **Inhalation - Risk Assessment**

A minimal risk level (MRL) of 1.7 ppm has been derived for “acute-duration” inhalation exposure (14 days or less) to Ammonia. The study involved 16 subjects exposed to Ammonia (50 ppm, 80 ppm, 110 ppm, or 140 ppm). The MRL is based on a LOAEL of 50 ppm for mild irritation to the eyes (6 subjects), nose (20 subjects), and throat (9 subjects) in humans exposed to Ammonia as a gas for 2 hours. The 1.7 ppm MRL was calculated  $(50 \text{ ppm} \div 30 [\text{uncertainty factor}] = 1.7; \text{uncertainty factor} = 10 [\text{to protect sensitive individuals}] \times 3 [\text{for use of a minimal LOAEL}] = 30)$ .<sup>79</sup> It should be noted that the Occupational Safety and Health Administration (OSHA) has established an 8-hour time weighted average exposure limit of 50 ppm (35 mg/m<sup>3</sup>) for Ammonia in the workplace.<sup>80</sup> Exposure to Ammonia shall not exceed the 50 ppm limit in any 8-h work shift of a 40-h work week.

Chronic occupational exposure (about 12.2 years) to low levels of airborne Ammonia (9.2 ppm) had no significant effect on pulmonary function or odor sensitivity in a group of workers at a soda ash factory, compared to a control group from the same factory that was not exposed to Ammonia.<sup>85</sup> The ATSDR derived a chronic inhalation MRL of 0.1 ppm for Ammonia from this study.<sup>9</sup> An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure. MRLs are based on non-cancer health effects only and are not based on a consideration of cancer effects. Derivation of the MRL is described below.

An MRL of 0.1 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to Ammonia. The MRL is based on a NOAEL of 9.2 ppm for sense of smell, prevalence of respiratory symptoms (cough, bronchitis, wheeze, dyspnea, and others), eye and throat irritation, and lung function parameters (forced vital capacity (FVC), forced expiratory volume at end of 1 second of forced expiration (FEV1), FEV1/FVC, forced expiratory flow at 50% of FVC (FEF50), and FEF at 75% of FVC (FEF75)) in humans exposed for an average of 12.2 years in a soda ash plant; no LOAEL was determined.<sup>85</sup> The cohort consisted of 52 workers and 35 controls (all males). The subjects were assessed on the first and last workday of their workweek. Spirometry was performed at the beginning and end of each work shift, so that each worker had four tests done. To determine the exposure levels, exposed and control workers were sampled (breathing zone air sample) over one work shift; the average sample collection period was 8.4 hours. Air samples were collected on sulfuric acid-treated silica gel adsorption tubes (tube holder attached to the collar).

Analysis of the results showed no significant differences in the prevalence of reported symptoms, but the exposed workers reported that exposure in the plant aggravated some of their reported symptoms (cough, wheeze, nasal complaints, eye irritation, and throat discomfort). There were no significant differences in baseline lung functions between exposed and control subjects. Analysis of each worker separately showed no significant relationship between the level of Ammonia exposure and changes in lung function. Also, when the workers were divided into groups of individuals that were exposed to low (< 6.25 ppm), medium (6.25 – 12.5 ppm), and high (> 12.5 ppm) Ammonia levels, no significant association was found between reporting of symptoms, decline in baseline function, or increasing decline in function over the work shift and exposure to Ammonia. Furthermore, no association was evident between increasing years of exposure and decreasing lung function. However, the power of the indices of both level and length of exposure is low because only eight workers were in areas with relatively high Ammonia exposure. The MRL was calculated by adjusting the mean time-weighted average (TWA) exposure concentration of 9.2 ppm for continuous exposure (8/24 hours x 5/7 days) and dividing by an uncertainty factor of 10 to protect all of the sensitive individuals. A modifying factor of 3 was added for the lack of reproductive and developmental studies.<sup>9</sup>

Based on occupational epidemiology studies, the EPA calculated a chronic inhalation reference concentration (RfC) of 0.5 mg/m<sup>3</sup>.<sup>2</sup> The critical effects in these studies were decreased lung function and respiratory symptoms.<sup>86,85,87,88</sup> The RfC is an estimate (with uncertainty ~ one order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

### **SUMMARY**

The safety of Ammonia and Ammonium Hydroxide as used in cosmetics is reviewed in this safety assessment. According to the *Dictionary*, both ingredients function as pH adjusters in cosmetic products. Additionally, Ammonia

functions as an external analgesic and fragrance ingredient and Ammonium Hydroxide functions as a denaturant in cosmetic products. Functioning as an external analgesic is not a cosmetic use and, therefore, the Panel did not evaluate safety in relation to that use in cosmetics. Additionally, the function of fragrance may be excluded from the purview of the Panel.

According to 2017 VCRP data, Ammonia is being used in 599 cosmetic products (mostly rinse-off products) and Ammonium Hydroxide is being used in 1354 cosmetic products (mostly rinse-off products). These uses of both ingredients are mostly in hair coloring products. The results of a concentration of use survey provided by the Council in 2017 indicate that the highest maximum cosmetic use concentration of Ammonia is 4.6% (in rinse off products (hair dyes and colors)) and the highest maximum cosmetic use concentration of Ammonium Hydroxide is 12.5% (in rinse off products (hair dyes and colors)). Regarding use concentrations in leave-on products, the highest maximum cosmetic use concentrations are 0.73% (Ammonia - in tonics, dressings, and other hair grooming aids) and 1.5% (Ammonium Hydroxide - in face and neck products (not spray)).

These two ingredients are indistinguishable from each other in aqueous formulation. Since the only cosmetic function of Ammonia applicable to this safety assessment is pH adjuster (which by default means aqueous formulations only) and Ammonium Hydroxide does not exist outside of water, regardless of which ingredient is added the final formulations will contain an equilibrium of molecular Ammonia and the ions of Ammonium Hydroxide in water. Thus, whether toxicity data is reported for Ammonia or Ammonium Hydroxide, it is applicable to both (as the test articles would have had this same equilibrium).

A large amount of metabolically-generated Ammonia is absorbed into the blood and is detoxified by the liver. The urea cycle (in liver), a cycle of biochemical reactions that produces urea from Ammonia, is the major pathway for Ammonia detoxification in terrestrial mammals. The normal range for Ammonia blood serum levels has been reported as 10-40  $\mu\text{mol/L}$ .

An acute oral  $\text{LD}_{50}$  of 350 mg/kg has been reported in a study involving rats dosed orally with Ammonia dissolved in water. Gastric lesions in rats have been observed after oral dosing (gavage) with 0.03% to 1% Ammonia and 1% and 3% Ammonium Hydroxide. The increase in gastric lesions observed was both concentration- and pH-dependent.

It has been noted that acute exposure data have demonstrated that injury to respiratory tissues is primarily due to Ammonia's alkaline (i.e., caustic) properties from the formation of hydroxide when it reacts with water. In acute inhalation toxicity studies, Ammonia was tested at concentrations ranging from 3.5 ppm (cats and rabbits, 1 h exposure) to 54,289 ppm (rats, 10-minute exposure). Exposure to the highest concentration resulted in hemorrhagic lungs, and increased respiratory fluid output was noted at the lowest concentration. In 10-minute exposure studies involving mice,  $\text{LC}_{50}$  values ranging from 8723 ppm to 10,150 ppm have been reported. In mice exposed to Ammonia (100 - 800 ppm) for 30 minutes, an  $\text{RD}_{50}$  of 303 ppm was reported. Within the range of concentrations tested (3440 ppm to 12,940 ppm) in 1-h exposure studies involving mice, the following effects have been observed: hepatic lesions, congestion, and necrosis; eye irritation; dyspnea; pneumonitis and atelectasis; histopathological changes in the lung (alveolar disruption and loss of septal continuity), and, in some cases, coma and death.

Exposure durations ranged from 10 minutes (14,170-55,289 ppm) to 1-4 h (3028-5053 ppm) in acute inhalation toxicity studies involving rats. For the 10-minute exposure,  $\text{LC}_{50}$  values were ~ 22,885 ppm (males) and ~31,430 ppm (females) (at highest exposure concentration) and ~14,141 ppm (males) and ~19,769 ppm (females) (at lowest exposure concentration). For the 1 h and 4 h exposures, the  $\text{LC}_{50}$ s were ~17,633 ppm and ~7068 ppm, respectively, and corneal opacity and signs of typical upper respiratory tract irritation were observed.

In short-term oral toxicity studies involving rats, doses of ~ 42 mg/kg/day for 8 weeks resulted in mucosal atrophy in the stomach antrum, and doses up to 1500 mg/kg/day for 35 days resulted in treatment-related changes in body weight, hematological findings, clinical biochemistry findings, and non-neoplastic histopathological findings.

Ammonia was evaluated at concentrations ranging from 0.6 ppm to 1306 ppm in short-term inhalation toxicity studies. The results of these studies indicate histopathological changes of respiratory tissues in several animal species (lung inflammation in guinea pigs and rats; focal or interstitial pneumonitis in monkeys, dogs, rabbits, and guinea pigs; pulmonary congestion in mice; thickening of nasal epithelium in rats and pigs; nasal inflammation or lesions in rats and mice) across different dosing regimens. In general, responses in respiratory tissues increased with increasing Ammonia exposure concentration.

Fatty changes of liver plate cells were seen in rats following continuous exposure to Ammonia (642 ppm) for 90 days. Mild congestion/degenerative changes in internal organs were reported for guinea pigs exposed to ~ 170 ppm Ammonia for 18 weeks. Damaged tracheal mucosae were observed in rats exposed repeatedly to Ammonia (100 ppm) for 12 weeks. Mild leukocytosis was noted in rats after exposure to 143 ppm, but not 43 ppm of Ammonia repeatedly for 3 months.

(25- or 60-minute exposures every 48 h). A low incidence of mortalities (13 of 515 rats and 4 of 15 guinea pigs) was observed in mice and guinea pigs exposed continuously to 671 ppm Ammonia (reported as Ammonium Hydroxide) for 90 days. However, there were no mortalities or treatment-related histopathological findings in rats, guinea pigs, rabbits, monkeys, or dogs exposed continuously to ~57.43 ppm for 114 days.

Enlarged adrenal glands were observed in rabbits that received 124 mg /kg/day Ammonia (as Ammonium Hydroxide) by gavage in water for 17 months.

In a developmental toxicity study involving pregnant rats exposed to Ammonia in the diet (4293 mg/kg/day; as the ammonium ion [read-across for Ammonia]) from gestation day 1 through day 21 of lactation, body weights of male and female offspring were reduced. Neither reproductive nor developmental toxicity were reported in a study in which female pigs were exposed (inhalation exposure) to ~7 ppm or ~35 ppm Ammonia from 6 weeks prior to breeding until day 30 of gestation. In an oral reproductive and developmental toxicity study on diammonium phosphate (identified as an appropriate read across material for developmental and reproductive toxicity and included herein to support the overall weight of evidence) involving rats in which embryos were treated *in vitro* and then implanted, a NOAEL of 1500 mg/kg/day (highest dose tested) was reported.

In the Ames test with and without metabolic activation, Ammonia was non-genotoxic in *S. typhimurium* strains and in *E. coli* strain WP2 uvr A. Without metabolic activation, it was non-genotoxic to *E. coli* strain Sd-4-73. An increased frequency of micronuclei (compared to controls) was observed in Swiss albino mice that received single i.p. doses of Ammonia (12, 25, or 50 mg/kg). The maximum number of micronucleated polychromatic erythrocytes was associated with mice that received the highest dose (50 mg/kg), and there was clear correlation of dose-yield effects. In another micronucleus test, groups of 10 (5 males, 5 females) ddY mice received single i.p. doses of 62.5, 125, 250 and 500 mg/kg ammonium chloride (read-across for Ammonia, or i.p. doses of 31.3, 62.5, 125, and 250 mg/kg ammonium chloride (4 injections within 24 h). Ammonium chloride was not genotoxic.<sup>6</sup>

Increased frequencies of chromosomal aberrations, sister chromatid exchanges, and mitotic index, with increasing duration of exposure were reported for workers who had been exposed to Ammonia in a fertilizer factory.

Ammonia (whether reported as Ammonia or Ammonium Hydroxide) was not carcinogenic in Swiss and C3H mice dosed orally. A statistically significant increase in the incidence of gastric cancer (70%) was observed in rats dosed orally with MNNG and 0.01% Ammonia, when compared to dosing with MNNG alone. In another study, the size, depth, and metastasis of MNNG-initiated tumors were enhanced in rats dosed orally with Ammonia (~42 mg/kg/day). There was no evidence of a tumorigenic effect in Kid: CFLP mice treated by gavage with ammonia dissolved in water alone at a dose of 42mg/kg/day of Ammonia for 4 weeks or with diethyl pyrocarbonate alone, but 9 of 16 mice treated with a combination of ammonium and pyrocarbonate developed lung tumors. In inbred HSL mice treated by gavage with ammonium ion + diethyl pyrocarbonate during pregnancy and lactation, lung tumors were not observed in mice treated during pregnancy or in the suckling mice of dams treated during lactation. Ammonium sulfate was classified as non-carcinogenic in rats in a study involving dietary concentrations up to 3% daily for 104 weeks. Neoplastic lesions were observed in this study, but were deemed not treatment-related because of the spontaneous occurrence of these lesions in the rat strain (F344/DuCrj) that was tested.

It has been reported that hyperammonemia (a metabolic disturbance characterized by an excess of Ammonia in the blood) is associated with neuronal cell loss and cerebral atrophy that lead to mental retardation and cerebral palsy in pediatric patients.

At a concentration of 0.01 mg/ml Ammonia, bovine lymphocyte viability was significantly reduced after 24 h and 48 h of incubation. In another study, the mitogenic response of lymphocytes was reduced after preincubation with Ammonia.

Guinea pigs exposed to 90 ppm Ammonia for 3 weeks developed a significant decrease in the cell-mediated immune response to challenge with a derivative of tuberculin.

No overt symptoms of neurological disorders were reported in guinea pigs or monkeys that were exposed to up to 1105 ppm Ammonia for 6 weeks.

The minimum concentration of Ammonia that caused an inflammatory reaction when applied (single application) to the skin of rats and mice (6 per species) was > 25% (rats) and = 25% (mice). In a skin irritation study in which groups of 4 rats, guinea pigs, and mice were injected intradermally with Ammonia (0.01 ml), the minimum concentration that caused a positive reaction was 0.05% in rats, mice, and guinea pigs. Ammonia (reported as Ammonium Hydroxide; 20% and 12%) was corrosive to the skin of rabbits, whereas the 10% concentration was not.

The application of a saturated aqueous solution of Ammonia (reported as Ammonium Hydroxide) to the skin of 16 subjects resulted in blister formation and skin irritation. In a study involving 110 subjects, Ammonia (reported as Ammonium Hydroxide; 1:1 aqueous solution) was applied to the skin and the inflammatory reaction observed was considered slight.

In rabbits, Ammonia (1 mg of Ammonium Hydroxide) was classified as an ocular irritant and 28.5% Ammonia (reported as Ammonium Hydroxide) induced corneal opacity. Additionally, Ammonia caused conjunctivitis in rabbits at concentrations of 1% to 10% (reported as Ammonium Hydroxide), but not 0.3%.

Microscopic damage to the gastric mucosa was observed in the stomachs of male rats exposed (ex vivo) to Ammonia (up to 60 mmol/l of Ammonium Hydroxide) for 15 minutes.

In various clinical reports, ocular, nasal, and throat irritation were observed in human subjects exposed to Ammonia in the 25 ppm to 700 ppm concentration range.

A retrospective study was performed to assess the association between petrochemical exposure and spontaneous abortion. Study participants included 2853 non-smoking women who had been pregnant at least once, 96 of whom had been exposed to unknown Ammonia concentrations. Data analyses did not indicate any effect on spontaneous abortion.

Results from an occupational exposure study indicated that female Palestinian hairdressers exposed to high levels of Ammonia in the workplace had higher neutrophilic airway inflammation when compared to a non-exposed control group.

## **DISCUSSION**

The Panel noted that Ammonia and Ammonium Hydroxide, well-known skin irritants, are indistinguishable from each other in aqueous formulation. Furthermore, since the only cosmetic function of Ammonia applicable to this safety assessment is pH adjuster (which by default means aqueous formulations only) and Ammonium Hydroxide does not exist outside of water, regardless of which ingredient is added, the final formulations will contain an equilibrium of molecular Ammonia and the ions of Ammonium Hydroxide in water. Thus, whether toxicity data are reported for Ammonia or Ammonium Hydroxide, these data are applicable to both (as the test articles would have had this same equilibrium).

The Panel addressed the use of chemicals for read-across, and determined that information reported for the following chemicals is appropriate for read-across to Ammonia and Ammonium Hydroxide: data on “ammonium ion” (reproductive and developmental toxicity, genotoxicity, and carcinogenicity data; counter-ion not identified) that are included in the ATSDR toxicological profile for Ammonia; diammonium phosphate (repeated dose (short-term) oral toxicity and reproductive and developmental toxicity data) that are included in the CIR Final Report on Phosphoric Acid and Its Salts and in an ECHA registration dossier on Ammonia; and data on ammonium chloride (genotoxicity data [micronucleus test]) and ammonium sulfate (oral carcinogenicity and chronic oral toxicity data) that are included also included in the ECHA dossier. Ammonium Hydroxide, diammonium phosphate, ammonium chloride, and ammonium sulfate are all low-formula-weight, inorganic ammonium salts. The Panel stated that, because the distribution and metabolism of the ammonium exposure from these salts should be essentially identical, information on diammonium phosphate, ammonium chloride, and ammonium sulfate is useful for evaluating the safety of Ammonia and Ammonium Hydroxide. The use of these chemicals for read-across is presented in Table 1.

Skin sensitization data are absent from the safety assessment. However, the Panel noted that these ingredients are corrosive, but there are no concerns relating to the sensitization potential of Ammonia or Ammonium Hydroxide. Therefore, the Panel determined that cosmetic products containing these ingredients should be formulated to be non-irritating, and that skin contact should be avoided to minimize irritation.

Lastly, the Panel recognized that there are reports of safety issues relating to chronic ingredient exposure experienced by hairdressers, but acknowledged that evaluation of occupational safety is not within the purview of the Panel. However, the Panel reviewed one such report in which hairdressers had signs of airway inflammation after exposure to Ammonia at concentrations up to 61 mg/m<sup>3</sup> in relatively small-sized salons with scarce ventilation. The Panel questioned the validity of this study in terms of any association between Ammonia inhalation exposure and airway inflammation, having noted that many chemicals are present in the salon environment.

## **CONCLUSION**

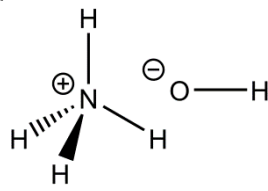
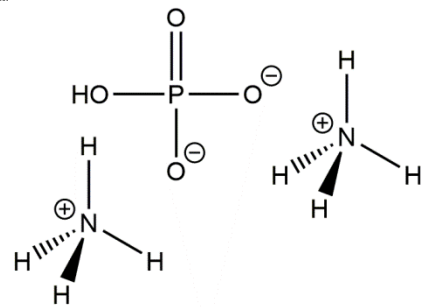
The CIR Expert Panel concluded that Ammonia and Ammonium Hydroxide are safe as used in hair dyes and colors and safe in cosmetics applied directly to the skin in the present practices of use and concentration described in the safety assessment when formulated to be non-irritating.

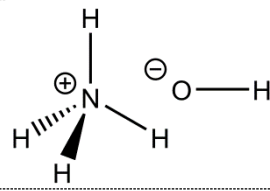
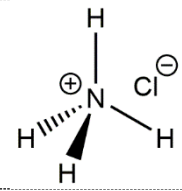


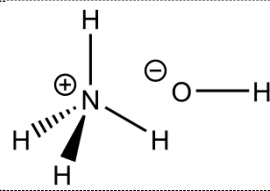
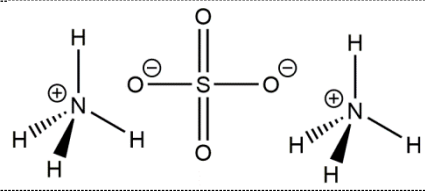


## TABLES

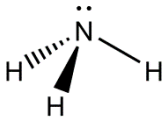
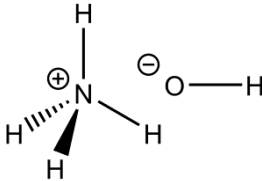
**Table 1.** Read-Across Justifications

Target Material		Read-Across Material
Name	Ammonia/Ammonium Hydroxide	diammonium phosphate
CAS No(s).	7664-41-7; 8007-57-6; 1336-21-6	7783-28-0
Structure		
read-across endpoints	<ul style="list-style-type: none"><li>• short-term toxicity – oral</li><li>• reproductive &amp; developmental</li></ul>	
justification	chemical properties, physical properties and metabolism are expected to be essentially identical for these two ammonium salts	
Examples:		
Formula weight (Da)	35.05	132.03
log K <sub>ow</sub> (estimated) <sup>11</sup>	-4.37 – 0.28 (dissolved NH <sub>3</sub> )	-2.85

Ammonia/Ammonium Hydroxide		ammonium chloride
CAS No(s).	7664-41-7; 8007-57-6; 1336-21-6	1448438-95-6; 12125-02-9
Structure		
read-across endpoints	<ul style="list-style-type: none"><li>• genotoxicity; <i>in vitro</i></li></ul>	
justification	chemical properties, physical properties and metabolism are expected to be essentially identical for these two ammonium salts	
Examples:		
Formula weight (Da)	35.05	53.49
log K <sub>ow</sub> (estimated) <sup>11</sup>	-4.37 – 0.28 (dissolved NH <sub>3</sub> )	-4.37

Ammonia/Ammonium Hydroxide		ammonium sulfate
CAS No(s).	7664-41-7; 8007-57-6; 1336-21-6	7783-20-2
Structure		
read-across endpoints	<ul style="list-style-type: none"><li>• chronic toxicity; oral</li><li>• carcinogenicity; oral</li></ul>	
justification	chemical properties, physical properties and metabolism are expected to be essentially identical for these two ammonium salts	
Examples:		
Formula weight (Da)	35.05	132.13
log K <sub>ow</sub> (estimated) <sup>11</sup>	-4.37 – 0.28 (dissolved NH <sub>3</sub> )	0.48

**Table 2. Definition, Idealized Structures, and Functions of the Ingredients in this Safety Assessment.** <sup>(1; CIR Staff)</sup>

Ingredient CAS No.	Definition & Idealized Structures	Function
Ammonia 7664-41-7 8007-57-6	<p>Ammonia is an inorganic gas that conforms to the formula:</p>  <p>(See also Ammonium Hydroxide)</p>	External Analgesics; Fragrance Ingredients; pH Adjusters
Ammonium Hydroxide 1336-21-6	<p>Ammonium Hydroxide is an inorganic base that conforms to the formula:</p>  <p>[In reality however, the solid, anhydrous salt does not exist. Instead, Ammonium Hydroxide is only present as an aqueous ion pair, the result of protic equilibration upon dissolution (not dissociation of a solid salt), in equilibrium with dissolved ammonia]</p>	Denaturants; pH Adjusters

**Table 3. Physical and Chemical Properties of Ammonia and Ammonium Hydroxide**

Property	Value	Reference
<b>Ammonia</b>		
physical form and/or color	Gas at room temperature; colorless	9
molecular weight (Daltons (Da))	17.03	9
water solubility (% w/w at 20°C)	33.1	9
Other solubility (%w/w at 25°C)	10 (absolute ethanol); 16 (methanol); soluble in chloroform and ether	9
density (g/L)	0.7710 (gas);	9
density (g/L at -33.5°C and 1 atm)	0.6818 (liquid); 0.7 (liquid)	9,10
vapor density (air = 1)	0.5967	9
specific gravity (g/L at 25°C)	0.747	9
melting point (°C)	-77.7	9,10
boiling point (°C)	-33.35	9,10
autoignition temperature (°C)	650	9
vapor pressure (atm at 20°C)	8.5	9
log K <sub>ow</sub> (estimated)	0.23	9
<b>Ammonium Hydroxide</b>		
Physical form	Colorless liquid	12
density (g/L at 20°C)	0.89801(28% aqueous)	9
Formula weight (Da)	35.05	10
melting point (°C)	-58 (25% aqueous)	12
boiling point (°C)	38 (25% aqueous)	12
vapor pressure (kPa at 20°C)	48 (25% aqueous)	12
log K <sub>ow</sub> (estimated)	-4.37	11

**Table 4. Frequency and Concentration of Use According to Duration and Type of Exposure.**<sup>16,17</sup>

	Ammonia		Ammonium Hydroxide	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)
<b>Totals/Conc. Range</b>	599	0.00002-4.6	1354	0.00028-12.5
<b>Duration of Use</b>				
<i>Leave-On</i>	7	0.00002-0.73	163	0.003-1.5
<i>Rinse off</i>	592	0.00015-4.6	1191	0.00028-12.5
<i>Diluted for (bath) Use</i>	NR	NR	NR	NR
<b>Exposure Type</b>				
<i>Eye Area</i>	1	NR	42	0.022-0.58
<i>Incidental Ingestion</i>	NR	NR	NR	NR
<i>Incidental Inhalation- Sprays</i>	3***	0.73*	6*	0.29-1.3*
<i>Incidental Inhalation- Powders</i>	3***	0.00002-0.14**	NR	0.45-1.5**
<i>Dermal Contact</i>	6	0.00002-0.14	159	0.0012-1.7
<i>Deodorant (underarm)</i>	NR	NR	NR	NR
<i>Hair - Non-Coloring</i>	10	0.00006-1.4	72	0.00028-3.6
<i>Hair-Coloring</i>	582	2.8-4.6	1104	2.5-12.5
<i>Nail</i>	1	0.00008-0.00075	3	0.003-1.2
<i>Mucous Membrane</i>	NR	NR	1	NR
<i>Baby Products</i>	NR	NR	NR	NR

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for Bath Product Uses.

\*It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.

\*\*It is possible that these products may be powders, but it is not specified whether the reported uses are powders.

\*\*\*Not specified whether a powder or spray, so this information is captured for both categories of incidental inhalation.

Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

**Table 5. Acute Oral Toxicity Studies**

Ingredient	Animals	Protocol	Results
Ammonia (0.03, 0.1, 0.3, 0.5, or 1%)	Male Wistar rats (groups of 6)	Administered by oral gavage	Minimal concentration at which gastric lesions observed was 0.03%. Over the range of concentrations administered, there was an increase in gastric juice pH and the ulcer index in an Ammonia concentration-dependent manner (mean pH: 4.38 to 8.45). <sup>42</sup>
Ammonia (dissolved in water)	Male Wistar rats (groups of 10)	Administered by gavage according to Organization for Economic Co-operation and Development (OECD) Guideline 401. Dosing followed by 14-day observation period	LD <sub>50</sub> (calculated) = 350 mg/kg. <sup>6,43,44</sup>
Ammonium Hydroxide (1% or 3%)	Male Sprague-Dawley rats (groups of 4 to 8)	Administered by gavage	Severe hemorrhagic lesions produced in a concentration-related manner. The lesion scores at 1% and 3% concentrations were 26.6 ± 9.3 mm <sup>2</sup> and 97.7 ± 8.3 mm <sup>2</sup> , respectively. The pH of 3% solution was 11.5. When this pH was decreased up to 7.0, by neutralizing with 0.1 N hydrochloric acid, the ulcerogenic activity of Ammonium Hydroxide was significantly mitigated at pH 10 and completely disappeared at pH 9. <sup>45</sup>

**Table 6. Acute Inhalation Toxicity**

Ingredient	Animals/Protocol	Results
Ammonia (21,400 ppm)	Mice. 30-minute exposure	Signs and symptoms included eye irritation (blinking and scratching), dyspnea, frothing, convulsions, excitation/escape behavior, coma, and death. Histopathology of the lungs of mice that died showed alveolar disruption and loss of septal continuity. <sup>24,50</sup>
Ammonia (8770-12,940 ppm)	Mice (groups of 20). 10-minute exposure	LC <sub>50</sub> = 10,150 ppm. <sup>47,46,48</sup>
Ammonia (8723-12,870 ppm)	Mice (groups of 20). 10-minute exposure	At 8,723 ppm, 25% of the animals died. At 12,870 ppm, 80% of the animals died. LC <sub>50</sub> = 10,096 ppm. <sup>24,46</sup>
Ammonia (3600-5720 ppm)	Mice. 1-h exposure	Nasal and eye irritation, followed by labored breathing, in all groups. Gross examination of surviving mice showed mild congestion of the liver at the intermediate (4550 ppm) and high (5720 ppm) concentrations. LC <sub>50</sub> = 4837 ppm (95% CI = 4409–5305 ppm). <sup>24,51,48</sup>
Ammonia (1190-4860 ppm)	ICR male mice (groups of 12). 1-h exposure	In animals that survived 14-day observation period, pathologic lesions included mild-to-moderate pneumonitis (dose-related severity), focal atelectasis in the lungs (4,860 ppm), and degenerative hepatic lesions (dose-related severity, 3,440–4,860 ppm). LC <sub>50</sub> = 4,230 ppm. <sup>24,52,48</sup>
Ammonia (4840 ppm)	Mice. 1-h exposure	Signs and symptoms included eye irritation (blinking and scratching), dyspnea, frothing, convulsions, excitation/escape behavior, coma, and death. Histopathology of the lungs of mice that died showed alveolar disruption and loss of septal continuity. <sup>24,53</sup>
Ammonia (3440 ppm)	Mice (groups of 12). 1-h exposure	Liver necrosis. <sup>52</sup>
Ammonia (92 mg/m <sup>3</sup> [~132 ppm] to 1243 mg/m <sup>3</sup> [~1785 ppm])	SPF mice of the OF1-ICO strain. Nose-only exposure for 45 minutes	Mice appeared more susceptible to ammonia in presence of dry air (RD <sub>50</sub> (exposure concentration producing a 50% decrease in respiratory rate) = 582 [407 ppm] and 732 mg/m <sup>3</sup> [547 ppm] in dry and wet air, respectively). <sup>24,56</sup>
Ammonia (100-800 ppm)	Male Swiss-Webster mice (groups of 4). 30-minute exposure	RD <sub>50</sub> = 303 ppm (95% confidence limits = 188–490 ppm). <sup>24,49,48</sup>
Ammonia (9870 mg/m <sup>3</sup> air [14,170 ppm] to 37,820 mg/m <sup>3</sup> air [54,289 ppm])	SPF-bred Wistar rats (5 males, 5 females/group). Exposures: 20,950 to 37,820 mg/m <sup>3</sup> air (10 minutes); 18,290 to 23,200 mg/m <sup>3</sup> air (20 minutes); 12,620 to 16,840 mg/m <sup>3</sup> air (40 minutes), and 9870 to 13,240 mg/m <sup>3</sup> air (60 minutes)	LC <sub>50</sub> (10-minute exposure) = 28,130 mg/m <sup>3</sup> air (40,300 ppm); LC <sub>50</sub> (20-minute exposure) = 19,960 mg/m <sup>3</sup> air (28,595 ppm); LC <sub>50</sub> (40-minute exposure) = 14,170 mg/m <sup>3</sup> air (20,300 ppm); and LC <sub>50</sub> (60-minute exposure) = 11,590 mg/m <sup>3</sup> air (16,600 ppm). Hemorrhagic lungs in animals that died. <sup>55</sup>

**Table 6. Acute Inhalation Toxicity**

Ingredient	Animals/Protocol	Results
Ammonia (9000-35,000 ppm)	Male Sprague-Dawley rats: 4 groups of 6 (9,000 to 26,000 ppm), 1 group of 8 (30,000 ppm), and 1 group of 4 (35,000 ppm). Exposure for 20 minutes in head-only exposure system	Lung edema increased in all groups. Dose-dependent increases in ocular irritation, lacrimation, and labored breathing. $LC_{50}$ (determined by probit analysis) = 23,672 ppm. <sup>57</sup>
Ammonia (9000 to 23,000 ppm)	Groups of 6 male Sprague-Dawley rats. Exposure for 20 minutes in head-only exposure system for 20 minutes	Peak inspiratory and expiratory flow decreased after exposure to 20,000 and 23,000 ppm. Weight loss, and increased total blood cell counts (white blood cells, neutrophils, and platelets) after exposure to 20,000 ppm. Morphological changes at histopathologic examination of lungs and trachea: alveolar, bronchial, and tracheal edema; epithelial necrosis, and exudate at 20,000 ppm. <sup>58</sup>
Ammonia (3028-14,044 ppm )	Male and female SPF-bred Wistar rats (Hsd Cpb:WU strain; 5 males, 5 females). Nose-only exposure to 9,222-14,044 ppm for 1 h and 3,028-5,053 ppm for 4 h.	Signs typical of upper respiratory tract irritation. No gross abnormalities in any organ or nasal passages were found at necropsy of surviving rats (2 weeks post-exposure). Rats that died had corneal opacity, collapsed lungs, nasal discharge, reddened larynx, and tracheal epithelial desquamation. $LC_{50}$ (1-h exposure) = 12,303 mg/m <sup>3</sup> [~17,633 ppm]. $LC_{50}$ (4-h exposure) = 4,923 mg/m <sup>3</sup> [~7068 ppm]. <sup>54</sup>
Ammonia (6210-9840 ppm)	Groups of 10 male CFE rats. 1-h exposure	Signs of eye and nasal irritation observed immediately, followed by labored breathing and gasping. Surviving animals exposed to the low concentration weighed less than controls on day 14, and gross examination showed mottling of the liver and fatty changes at the two highest concentrations. $LC_{50}$ = 7338 ppm (95% CI = 6822–7893 ppm). <sup>24,51-48</sup>
Ammonia (431, 1436, and 4307 ppm)	Rats. Inhalation exposure for 5, 15, 30, or 60 minutes	Decrease in static muscular tension and other sublethal effects. <sup>48</sup>
Ammonia (1436, 4307, and 6814 ppm)	White rats (number not stated). Inhalation exposure for 5, 15, 30, or 60 minutes	Dyspnea, irritation of respiratory tract and eyes, cyanosis of extremities, and increased excitability. <sup>48</sup>
Ammonia (92 mg/m <sup>3</sup> [~132 ppm] to 1243 mg/m <sup>3</sup> [~1785 ppm])	Groups of 4 male specific pathogen free (SPF) Wistar rats of the Hsd Cpb:WU (SPF) strain. Nose-only exposure for 45 minutes	$RD_{50}$ = 972 and 905 mg/m <sup>3</sup> (corresponding to ~1396 and ~1299 ppm, respectively) in rats in dry and wet air, respectively. <sup>24,56</sup>
Ammonia (144 ppm)	Rats (number not stated). Inhalation exposure for 5, 10, 15, 30, or 60 minutes	No effects. <sup>48</sup>
Ammonia (5,200-12,800 ppm)	Rabbits. 1-h exposure	Average survival: 18 h (gassed after cannulation), 33 h (gassed before cannulation). 2- to 3-fold increase in production of respiratory tract fluid. No change in water content of lungs. Increased blood hemoglobin. Increased plasma lipids. <sup>24</sup>

**Table 6. Acute Inhalation Toxicity**

Ingredient	Animals/Protocol	Results
Ammonia (10,360 ppm, average)	Rabbits. 1-h exposure	Congestion of respiratory tract tissues. <sup>24</sup>
Ammonia (50 ppm and 100 ppm)	16 New Zealand White rabbits. Inhalation Exposure for 2.5 h to 3 h	Significant decrease in rate of respiration. <sup>48</sup>
Ammonia (3.5 ppm and 8.7 ppm)	54 rabbits. Exposure for 1 h	Increased respiratory tract fluid output by 2- to 3-fold. No appreciable effect on water content of respiratory tract tissues. Transient decrease in blood hemoglobin. Lipemia also observed. <sup>48</sup>
Ammonia (5,200-12,800 ppm)	Cats. 1-h exposure	Average survival: 18 h (gassed after cannulation), 33 h (gassed before cannulation). 2- to 3-fold increase in production of respiratory tract fluid. No change in water content of lungs. Increased blood hemoglobin. Increased plasma lipids. <sup>47,65</sup>
Ammonia (10,360 ppm, average)	Cats. 1-h exposure	Congestion of respiratory tract tissues. <sup>47,65</sup>
Ammonia (1,000 ppm)	20 cats. 10-minute exposure	Biphasic course of respiratory pathology. Effects at 24 h post-exposure included severe dyspnea, anorexia, and dehydration; rhonchi and coarse rales evident upon auscultation. Gross pathology revealed varying degrees of congestion, hemorrhage, edema, interstitial emphysema, and collapse of the lungs at all time points. Pulmonary resistance increased throughout the study. <sup>48,64</sup>
Ammonia (3.5 ppm and 8.7 ppm)	18 cats. Exposure for 1 h	Increased respiratory tract fluid output by 2- to 3-fold. No appreciable effect on water content of respiratory tract tissues. Transient decrease in blood hemoglobin. <sup>48</sup>

**Table 7. Short-Term and Subchronic Toxicity Studies**

Ingredient	Animals	Protocol	Results
Short-term Oral Studies			
Ammonia (0.01% in drinking water)	Rats (groups of 36)	5 groups initially received tap water : group 1 (for 7 weeks and 4 days), group 2 (7 weeks), group 3 (6 weeks), group 4 (4 weeks), and group 5 (0 water before Ammonia dosing). Groups then received Ammonia at dose of ~ 42 mg/kg/day for 8 weeks.	No mucosal lesions at macroscopic or microscopic examination. Mucosal atrophy in stomach antrum and enlargement of proliferative zone in antral and body mucosa. <sup>67</sup>
diammonium phosphate (read-across for Ammonia, 17.9% N and 46.86% P <sub>2</sub> O <sub>5</sub> equivalent)	Groups of Crj: CD(SD) rats (5 males, 5 female/group)	Administered by gavage daily (doses of 0, 250, 750, and 1500 mg/kg/day, 7 days/week) for 35 days	Clinical signs were not observed, and none of the animals died. However, there were treatment-related changes in body weight, hematological findings, clinical biochemistry findings, and non-neoplastic histopathological findings. Histological examination of stomachs revealed some submucosal inflammation at all doses, but this change was not dose-dependent and was not statistically significant at the low dose. NOAEL for general toxicity of diammonium phosphate = 250 mg/kg/day; LOAEL for general toxicity of diammonium phosphate = 750 mg/kg/day. <sup>6,48</sup>
Short-term Inhalation Studies			
Ammonia (~1306 ppm)	Rats	5 days/week (8 h/day)	Exposure tolerated for 42 days. <sup>73</sup>
Ammonia (~223 ppm or ~1105 ppm)	Sprague-Dawley and Long-Evans rats (males and females, groups of 15); Male New Zealand albino rabbits (groups of 3); Princeton-derived guinea pigs (males and females, groups of 15); Male squirrel monkeys ( <i>Saimiri sciureus</i> , groups of 3); Beagle dogs (groups of 2)	Exposure 5 days per week (8 h/day) for 6 weeks	Lung effects: Gross necropsies normal. Focal pneumonitis in 1 of 3 monkeys at 223 ppm. Nonspecific lung inflammation in guinea pigs and rats, but not in other species at 1105 ppm. Upper respiratory tract effects: mild to moderate dyspnea in rabbits and dogs exposed to 1105 ppm during week 1 only; no indication of irritation after week 1. Nasal turbinates not examined for gross or histopathologic changes <sup>2,40,73</sup>
Ammonia (1086 ppm)	Rats, squirrel monkeys, and guinea pigs (number per species not stated)	Inhalation exposure 5 days per week (8 h/day) for 6 weeks	No fatty changes of liver plate cells. No pathological changes in kidney. <sup>9</sup>
Ammonia (653 ppm)	Rats (number not stated)	Continuous inhalation exposure for 25 days	Nearly 64% lethality. <sup>9</sup>
Ammonia (~653 ppm)	Sprague-Dawley or Long-Evans rats (males and females, 15 to 51/group)	Inhalation exposure for 65 days	Lung effects: Focal or diffuse interstitial pneumonitis in all animals. Upper respiratory tract effects: Dyspnea and nasal irritation/discharge. <sup>2,73</sup>
Ammonia (650 ppm; Ct [product of concentration and exposure time (ppm-h)] = 390,000 and 1,014,000)	51 rats	Continuously for 65 days	32 of 51 rats dead by day 25 (390,000 ppm-h); 50 of 51 rats dead by day 65 (1,014,000 ppm-h). <sup>47,73</sup>



**Table 7. Short-Term and Subchronic Toxicity Studies**

Ingredient	Animals	Protocol	Results
Ammonia (500 ppm)	27 male rats	Continuous inhalation exposure for up to 8 weeks	After 3 weeks, nasal irritation and inflammation of upper respiratory tract, but no effects observed in bronchioles and alveoli. No lesions observed at 8 weeks. <sup>48,74</sup>
Ammonia (250 ppm)	F344 rats (6/sex/group)	Exposure in inhalation chamber for 35 days	Increased thickness of nasal epithelium (3 to 4 times) and nasal lesions at 150 ppm. <sup>2,72</sup>
Ammonia (221 ppm; Ct [ppm-h] = 53,040)	Rats, guinea pigs, rabbits, squirrel monkeys, and beagle dogs (number per species not stated)	5 days per week (8 h per day) for 6 weeks	No effect. <sup>47,73</sup>
Ammonia (10 or 150 ppm)	Sherman rats (5/sex/group)	Inhalation exposure from bedding for 75 days	Increased thickness of nasal epithelium (3 to 4 times) and nasal lesions at 150 ppm. <sup>2,48,72</sup>
Ammonia (50 or 90 ppm)	Male Wistar rats (8-14 per group)	Inhalation exposure continuously for 50 days	None of the animals died and there were no treatment-related effects. <sup>48,76</sup>
Ammonia (12% solution)	50 male White albino mice	Vapor exposure 6 days per week (15 minutes/day) for 4, 5, 6, 7, or 8 weeks	Nasal mucosa adversely affected. Histological changes progressed from weeks 4–8 from crowding of cells forming crypts and irregular arrangements to epithelial hyperplasia, patches of squamous metaplasia, loss of cilia, and dysplasia of the nasal epithelium. One animal that had loss of polarity of the epithelium, hyperchromatism, and mitotic figures with an intact basement membrane also had a carcinoma <i>in situ</i> in one nostril. At week 8, one mouse had an invasive adenocarcinoma of the nasal mucosa. Histochemical results were also abnormal. <sup>2,71</sup>
Ammonia (78 ppm, 271 ppm, and 711 ppm)	Groups of 10 male Swiss mice	Exposure for 4, 9, or 14 days (6 h/day)	No clinical signs of toxicity were noted for mice exposed to ammonia. Rhinitis and pathologic lesions with metaplasia and necrosis were seen only in the respiratory epithelium of the nasal cavity of mice inhaling 711 ppm, the severity of the lesions increased with duration of exposure, ranging from moderate on day 4, severe on day 9, to very severe on day 14. No lesions were seen in the controls or in mice inhaling the 78 ppm. No effects were seen at 271 ppm, even after 9 days of exposure. <sup>24,68</sup>
Ammonia (303 ppm)	Groups of 16 to 24 male Swiss Webster mice	Exposure for 5 days (6 h/day)	Histopathological findings, which were confined to the respiratory epithelium of the nasal cavity, included minimal exfoliation, erosion, ulceration, and necrosis; moderate inflammatory changes; and slight squamous metaplasia. <sup>24,69</sup>

**Table 7. Short-Term and Subchronic Toxicity Studies**

Ingredient	Animals	Protocol	Results
Ammonia (20 ppm)	Swiss albino mice (males and females, groups of 4)	Exposure for 7, 14, 21, 28, or 42 days	Lung congestion, edema, and hemorrhage observed after 42 days. <sup>2,70</sup>
Ammonia (170 ppm; Ct [ppm-h] =30,600 to 91,800)	12 male Guinea pigs	5 days per week (6 h per day) for 6 weeks	No histopathologic changes. <sup>47,81</sup>
Ammonia (50 ppm)	Guinea pigs (males and females, groups of 6)	Exposure for 42 days	Lung congestion, edema, and hemorrhage. <sup>2,70</sup>
Ammonia (20 ppm)	Guinea pigs (males and females, groups of 2)	Exposure for 7, 14, 21, 28, or 42 days	Lung congestion, edema, and hemorrhage after 42 days. <sup>2,70</sup>
Ammonia (100 ppm [average range = 20 to 203 ppm; Ct [ppm-h] =100,800) alone and with corn starch dust	Yorkshire-Landrace pigs (groups of 6)	Continuously for 6 weeks	Tracheal damage (thickened tracheal epithelium [50 to 100% increase] and goblet cells reduced) at end of week 2 in animals exposed to 100 ppm (33,600 ppm-h) without dust. Changes more prominent by week 6. Conjunctival irritation more severe in pigs exposed to ammonia and corn starch dust, persisting for 2 weeks. <sup>2, 47,135</sup>
Ammonia (10 ppm and 50 to 150 ppm; Ct [ppm-h] = 42,000 to 126,000)	Duroc Pigs (groups of 36)	Continuously for 5 weeks	Excessive nasal, lacrimal and mouth secretions at 50, 100, and 150 ppm; more pronounced at 100 and 150 ppm, gradually diminishing over 1-2 weeks. No histopathologic changes in nasal turbinates or lung. <sup>6,47,75</sup>
Ammonia (12, 61, 103, or 145 ppm)	Duroc pigs (males and females, groups of 9)	Exposure for 5 weeks	Excessive nasal, lacrimal, and mouth secretions, and increased frequency of cough at 103 and 145 ppm. <sup>2,75</sup>
Ammonia (5 ppm [range = 0 to 7 ppm] to 100 ppm [range = 90 to 112 ppm])	Belgian Landrace pigs (groups of 7)	Nasal lavage technique. 6-day exposure in chamber	No-observed-effect value for Ammonia-induced somatic growth inhibition < 25 ppm. Nasal irritation down to 25 ppm. Conjunctival irritation observed in 4 pigs exposed to 100 ppm. Lethargy in groups exposed to 25, 50 and 100 ppm for 2 to 3 days after placement in chamber. <sup>78</sup>
Ammonia (0.6, 10, 18.8, or 37 ppm)	Pigs (different breeds, groups of 24)	Inhalable dust exposure for 5 weeks	No increase in incidence of respiratory diseases. <sup>2, 77</sup>
Ammonia (~1.8, ~3.9, ~7.3, or ~14.2 ppm)	Pigs (different breeds, groups of 24)	Inhalable dust exposure for 5 weeks	No increase in incidence of respiratory diseases. <sup>2, 77</sup>
Subchronic Inhalation Studies			
Ammonia (642 ppm)	Rats (number not stated)	Continuous exposure for 90 days	Fatty changes of liver plate cells. <sup>9</sup>
Ammonia (43 ppm or 143 ppm)	White rats (number not stated)	Inhalation exposure for 3 months (25- or 60-minute exposures every 48 h)	Mild leukocytosis after exposure to 143 ppm. No adverse effects after exposure to 43 ppm. <sup>48</sup>
Ammonia (100 ppm)	Rats	Inhalation exposure 5 days per week (5 h/day) for 12 weeks	Damaged tracheal mucosae. <sup>47,82</sup>

**Table 7. Short-Term and Subchronic Toxicity Studies**

Ingredient	Animals	Protocol	Results
Ammonia (~170 ppm)	12 male guinea pigs (additional 6 were controls)	Inhalation exposure 5 days per week (6 h/day) for 18 weeks	No significant findings after 6 and 12 weeks of exposure. Results at 18 weeks were: relatively mild congestion of the liver, spleen, and kidneys; degenerative changes in adrenal glands; hemosiderosis in spleen (indicative of hepatotoxicity); and cloudy swelling in epithelium of proximal kidney tubules, with albumin precipitation in lumen. <sup>47,81</sup>
Ammonium Hydroxide (671 ppm)	515 rats and 15 guinea pigs	Inhalation exposure continuously for 90 days	13 rats and 4 guinea pigs died. <sup>48</sup>
Ammonium Hydroxide (~57.43 ppm)	15 Sprague-Dawley/Long-Evans rats (males and females), 15 Princeton-derived guinea pigs (males and females), 3 male New Zealand albino rabbits, 3 male squirrel monkeys, and 2 purebred male beagle dogs	Inhalation exposure continuously for 114 days	No mortalities or signs of toxicity. Necropsy observations were normal and there were no treatment-related histopathological findings. <sup>73</sup>

**Table 8. Developmental and Reproductive Toxicity Studies**

Ingredient	Animals/Embryos	Protocol	Results
In Vitro/In Vivo Study			
Ammonium ion (read-across for Ammonia, 38 to 300 $\mu\text{mol/l}$ )	Mouse embryos (conceived in vivo)	Embryos cultured in modified mouse tubal fluid medium (mMTF) or mMTF supplemented with 300 $\mu\text{mol/L}$ ammonium ion for 48, 69, or 93 h before being transferred to pseudo-pregnant mouse dams	Examination on gestational day 15 showed apparent relationship between the duration of exposure and the incidence of exencephaly. Increased incidence of exencephaly with increased ammonium concentration (38–300 $\mu\text{mol/L}$ ) and decreased percentage of implantation sites with increased ammonium concentration. <sup>89</sup>
Oral Studies			
Ammonium ion (read-across for Ammonia)	Pregnant rats	Feeding with ammonium ion in the diet (4293 mg ammonium/kg/day) from gestation day 1 through day 21 of lactation	Growth of rats exposed during pregnancy and lactation was significantly lower than in controls until approximately day 60. Body weights of offspring reduced by 25% (males) and 16% (females). <sup>9,90</sup>
diammonium phosphate (read-across for Ammonia, 17.9% $\text{NH}_4$ and 46.86% $\text{P}_2\text{O}_5$ equivalent)	Groups of Crj: CD(SD) rats (5 males, 10 females [reproductive subgroup])	Administered by gavage daily (doses of 0, 250, 750, and 1500 mg/kg/day) for, at most, 28 days (males) and 53 days (females).	No treatment-related deaths and no signs of overt clinical toxicity. Body weight gain was reduced during the first week of gestation (82% of control) in females dosed with 1500 mg/kg/day, but returned to control levels for remainder of study. Mating performance and fertility were unaffected by treatment, and parental treatment had no apparent effect on the offspring to day 4 of age. NOAEL for reproductive and developmental toxicity = 1500 mg/kg/day; LOAEL not identified. <sup>6,44</sup>
Inhalation Study			
Ammonia (7 ppm or 35 ppm)	Groups of 40 gilts (Yorkshire x Hampshire x Chester White)	Exposure for 6 weeks (7 ppm or 35 ppm). Exposure to ~7 ppm or ~35 ppm from 6 weeks prior to breeding until day 30 of gestation	No statistically significant differences in ovarian or uterine weights after 6 weeks of exposure. After exposure from 6 weeks prior to breeding until day 30 of gestation, no statistically significant differences in age at puberty, number of live fetuses, fetal length, or fetus-to-corpus luteum ratio compared to pigs exposed to only about 7 ppm. No unexposed controls were included in this study. <sup>91</sup>

**Table 9. Carcinogenicity and Tumor Promotion Studies**

Ingredient	Animals	Protocol	Results
Oral Studies			
Ammonia (dissolved in water)	Kid: CFLP mice	Development of lung tumors can be observed in Kid: CFLP mice treated intragastrically with diethyl pyrocarbonate and ammonia. The lung tumors may result from a carcinogenic substance, supposedly urethane, formed in vivo from diethyl pyrocarbonate in the presence of ammonia. Animals treated with 200 mg/kg diethyl pyrocarbonate and or 42 mg/kg Ammonia dissolved in water (8.4 mg/ml) by stomach tube twice per week for 4 weeks. Necropsy at 20 weeks after first treatment. Number of tumors counted under dissecting microscope.	No evidence of a tumorigenic effect was found in mice treated by gavage with ammonia dissolved in water alone at a dose of 42 mg Ammonia/kg/day for 4 weeks or with diethyl pyrocarbonate alone, but 9 of 16 mice treated with a combination of ammonium and pyrocarbonate developed lung tumors. <sup>93</sup>
Ammonium Hydroxide	Swiss and C3H mice	Exposure of mice to 193 mg ammonium/kg/day, as Ammonium Hydroxide (in drinking water), for 2 years	No carcinogenic effects, and did not affect spontaneous development of breast cancer (adenocarcinoma), which is common to C3H female mice. <sup>44, 48,94</sup>
Ammonium ion (read-across for Ammonia, and diethyl pyrocarbonate)	Groups of 16 inbred HSL mice	Exposure (by gavage) during pregnancy and lactation	No lung tumors on the surfaces of the lungs of mice treated during pregnancy or the suckling mice of dams treated during lactation. <sup>95</sup>
Ammonium Sulfate (read-across for Ammonia)	Groups of 100 F344/DuCrj rats (50 males and 50 females per group)	Dietary concentrations of 0%, 1.5%, and 3% daily for 104 weeks	<u>Survival rates:</u> males - 88% (controls), 78% (1.5% group), and 76% (3% group); females - , 76% (controls), 80% (1.5% group), and 80% (3% group) <u>gross finding:</u> massive, nodular or focal lesions suggestive of neoplastic change <u>neoplastic lesions</u> (not treatment-related; occur spontaneously in rats of this strain): C-cell adenomas/adenocarcinomas in the thyroids, fibroadenomas/adenomas/adenocarcinomas in mammary glands, adenomas/adenocarcinomas in pituitary glands, interstitial cell tumors in testes, and endometrial stromal polyps in uteri <u>non-neoplastic lesions:</u> the incidence of chronic nephropathy was statistically significantly increased in low-dose males No evidence of long-term carcinogenic activity. <sup>84</sup>

**Table 9. Carcinogenicity and Tumor Promotion Studies**

Ingredient	Animals	Protocol	Results
Ammonium Sulfate (read-across for Ammonia)	Groups of 10 F344/DuCrj rats (male and female)	Dietary concentrations of 0%, 0.1%, 0.6%, and 3% for 52 weeks	Neoplastic lesions reported included malignant pheochromocytoma of the adrenal gland in males of the 3% dietary group, 2 adenomas in the anterior pituitary of females of the 3% dietary group, and uterine endometrial stromal polyp in a female control rat. <sup>6</sup>
Inhalation Study			
Ammonia (12% solution)	10 male mice	Vapor exposure 6 days per week (15 minutes/day) for 4, 5, 6, 7, or 8 weeks	Histological changes progressed (weeks 4 to 8) from crowding of cells forming crypts and irregular arrangements to epithelial hyperplasia, patches of squamous metaplasia, loss of cilia, and dysplasia of the nasal epithelium. One mouse had a carcinoma <i>in situ</i> in 1 nostril. At week 8, 1 mouse with invasive adenocarcinoma of the nasal mucosa. Authors noted that prolonged exposure to Ammonia may interfere with normal protective reflexes of the respiratory nasal mucosa, resulting in the accumulation of particulate matter initiating or promoting a neoplastic process. <sup>71</sup>
Tumor Promotion			
Ammonia (dissolved in water)	Rats	Rats pretreated with the initiator <i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine (MNNG) in drinking water for 4 weeks, prior to receiving 0.01% Ammonia solution in drinking water for 24 weeks	Statistically significantly greater incidence of gastric cancer (70% of rats) and number of tumors per tumor-bearing rat (2.1) than rats that received only MNNG and tap water (31% and 1.3 tumors/rat). <sup>48,96</sup>
Ammonia	Rats	Rats pretreated with MNNG prior to dosing with Ammonia (~ 42 mg/kg/day)	The size, depth, and metastasis of the MNNG-initiated tumors enhanced in rats dosed with Ammonia. <sup>97</sup>

**Table 10. Dermal Irritation Studies**

Ingredient	Animals/Subjects/Cells	Protocol	Results
Skin Irritation Studies			
<b><u>In Vitro Studies</u></b>			
Undiluted Ammonium Hydroxide (30% active material in neat substance)	Reconstructed human skin cultures	Test substance applied topically to stratum corneum surface of cultures. Skin culture damage or cytotoxicity measured as decreased 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) vital dye metabolism. In time-course experiments, the time (in minutes) of test material exposure eliciting a 50% reduction of MTT metabolism (i.e., $t_{50}$ value) was calculated.	Histologic examination of the cultures indicated gradations of epidermal necrosis quantitated using a specially designed grading scale, which correlated well with the corrosivity of treatment chemicals and cytotoxicity measurements. Ammonium Hydroxide (30% active in neat substance) was classified as corrosive ( $t_{50}$ = 0.90 minutes). <sup>118</sup>

**Table 10. Dermal Irritation Studies**

Ingredient	Animals/Subjects/Cells	Protocol	Results
<b><u>Animal Studies</u></b>			
Ammonia	Wistar rats (3 males, 3 females) and ddY mice (3 males, 3 females)	Test solutions (1 ml/kg or 1 g/kg) applied once, unoccluded, to shaved skin of the back. Area of application was 3 x 4 cm for rats and 1 x 2 cm for mice. Distilled water control. Test sites observed for inflammatory reactions for 1 week after application.	Minimum concentration of Ammonia that caused a positive reaction was >25% (minimum amount = >250 mg/kg) in rats and 25% (minimum amount = 250 mg/kg) in mice. <sup>119</sup>
Ammonia	Wistar rats (4), Hartley guinea pigs (4), and ddY mice (4)	Injected intradermally with test solutions (0.01 ml) at 4 spots on shaved dorsal skin. Saline served as the control. The test sites were evaluated for skin irritation for up to 1 week after application.	The minimum concentration that resulted in a positive reaction was 0.05% in rats (minimum amount = 25 µg/kg), mice (minimum amount = 250 µg/kg), and guinea pigs (minimum amount = 12.5 µg/kg). <sup>119</sup>
Ammonium Hydroxide (10% and 20%)	Groups of 3 New Zealand Albino rabbits	Each concentration (0.5 ml) applied to the skin (2 replicates at each dose)	Results positive for skin corrosion at 20% concentration. Negative results at 10% concentration. <sup>20,44</sup>
Ammonium Hydroxide (10% and 12% aqueous)	Female Albino New Zealand White rabbits (groups of 3)	Each solution (0.1 ml) applied, under an occlusive patch (1" x 1"), to the skin for 4 h. There were 3 rabbits per dose, with 2 replicates per rabbit at each concentration.	The 12% solution was corrosive to the skin, but the 10% solution was not. <sup>6</sup>
<b><u>Human Studies</u></b>			
Ammonium Hydroxide (saturated aqueous solution)	16 subjects (10 men, 6 women)	Applied (via a chamber) to middle of ventral aspect of forearm	Formation of a well-defined, sub-epidermal blister (positive reaction) observed within a few minutes of chamber application; skin irritation observed in all subjects. <sup>120</sup>
Ammonium Hydroxide (1:1aqueous solution)	110 subjects	Test substance (0.5 ml) placed in 8 mm well drilled in acrylic plastic block (3 x 3 x 1 cm) that was strapped to the skin. Block (used to measure minimal blistering time (MBT, indicator of cutaneous irritability, defined as total exposure in well that results in a single bulla, occupying the total area of contact)).	MBT ranged from 3 to 57 minutes. Inflammatory reaction considered slight; healing was rapid and without scarring. Intensity of the dermatitis provoked by a 24-h exposure to sodium lauryl sulfate was strongly correlated with the MBT. <sup>121</sup>
Ammonium Hydroxide solution (50% solution)	Young adults and older adults (number not stated)	Acrylic plastic block with 14 mm well loosely strapped to skin. Well was then filled with 0.5 ml of freshly prepared 1:1 aqueous solution of Ammonium Hydroxide. Site was examined at 30-minute intervals, and blistering response was measured.	Mild discomfort during procedure. The initial response, characterized by the appearance of tiny follicular vesicles, occurred more quickly in older adults. The time required to produce a full blister was greatly prolonged in the aged. <sup>122</sup>

**Table 11. Ocular Irritation Studies**

Ingredient	Animals/Cells	Test Protocol	Results
<b><u>In Vitro</u></b>			
Ammonium Hydroxide	Human corneal endothelial cell cultures	<sup>51</sup> Cr-release assay. Performed by loading the cells with isotope, incubating the cells with Ammonium Hydroxide, and measuring the isotope that was recovered in the medium.	Severe ocular irritant (ED <sub>50</sub> = 3.9 x 10 <sup>-3</sup> M). <sup>123</sup>
<b><u>Animal</u></b>			
Ammonia (15, 32, 310, or 1157 ppm vapor concentrations)	Rats (CrI:COBS CD(SD) strain)	In phase 1 of study, groups of 8 rats exposed for 24 h. In phase 2, groups of 14 rats exposed for 3 or 7 days.	No clinical signs or evidence of irritation to the eyes or mucous membranes. No histologic differences in tracheal or lung sections between control and experimental groups. <sup>24,40</sup>
Ammonium Hydroxide	Rabbits (number not stated)	Instillation of test substance (1 mg) followed by ocular rinsing	Ocular irritant. <sup>44</sup>
Ammonium Hydroxide (28.5%)	Rabbits (number not stated)	Brief exposures (2 seconds). Volume instilled not stated	Corneal opacity. <sup>9,124</sup>
Ammonium Hydroxide (0.3%, 1%, 2.5%, and 10%)	New Zealand albino rabbits (groups of 6)	Draize test. Test substance (0.1 ml) instilled into the eye. In 1 group, eyes rinsed after instillation	Conjunctivitis (at 1% to 10%, but not at 0.3%). Ammonium Hydroxide (10%) produced pannus in 5/6 unwashed rabbit eyes and 2.5% produced pannus in 1/6 unwashed and 6/6 washed eyes. Ammonium Hydroxide at 1% produced pannus in 3/6 washed eyes. Keratoconus was produced by 10% Ammonium Hydroxide in 4/6 unwashed eyes and 2/6 washed eyes and 2.5% produced keratoconus in 2/6 unwashed eyes. Ammonium Hydroxide (10%) caused corneal opacities within 1 h of instillation. <sup>125</sup>
Ammonium Hydroxide (prepared with 3% Ammonia)	3 New Zealand White Albino Rabbits	Draize test. Test substance (100 µl) instilled into eye	Conjunctivitis (score = 3 at 96 h; mean maximum Draize score = 3), chemosis (score = 3 at 96 h; mean maximum score = 4), iritis (score = 1; mean maximum Draize score = 2), corneal opacity (score = 4; mean maximum Draize score = 4), and mean surface of corneal damage (70% corneal damage; mean maximum Draize value = 100%). Risk of serious damage to the eyes. <sup>126</sup>



**Table 12. Other Clinical Reports**

Ingredient	Number of Subjects	Protocol	Results
Inhalation Exposure			
Ammonia (700 ppm)	Number of subjects not available	Not available	Eye irritation. <sup>130</sup>
Ammonia (500 ppm)	Number of subjects not available	30-minute exposure	Variable lacrimation. <sup>130</sup>
Ammonia (500 ppm)	Number of subjects not available	30-minute exposure	Increased blood pressure and pulse rate. <sup>130</sup>
Ammonia (500 pm)	Number of subjects not available	30-minute exposure	Nasal and throat irritation, increased minute volume, and cyclic pattern of hyperpnea. <sup>130</sup>
Ammonia (500 ppm)	7 men	30-minute exposure	Increase in ventilation minute volume of 50-250%, accompanied by cyclic increase in respiratory rate. Irritation of the nose and throat. No significant change in nitrogen or urea in blood and urine. No significant change in serum nonprotein nitrogen. <sup>131</sup>
Ammonia (500 ppm)	7 subjects	30-minute exposure via face mask	Ventilation minute volume increased 50 to 250% over pre-exposure values. Respiratory minute volumes fell below pre-exposure levels at termination of exposure. <sup>47,131</sup>
Ammonia (101 to 335 ppm)	Number of subjects not available	20-minute exposure	Decrease in exercise ventilation minute volume at 151-335 ppm, related either to a decrease in respiratory rate (at 151 ppm) or tidal volume (at 205 and 335 ppm); no significant effects at 101 ppm. <sup>47,132</sup>
Ammonia (50 to 140 ppm)	16 subjects	2-h exposure. Testing repeated after a 1-week interval.	110 ppm tolerable for all subjects. 140 ppm intolerable at 1 h (4 subjects) and at 2 h (4 subjects). No significant increase in vital capacity, forced expiratory volume at end of 1 second of forced expiration (FEV <sub>1</sub> ), or forced inspiratory volume inhaled at end of 1 <sup>st</sup> second of forced inspiration (FIV <sub>1</sub> ). Lowest-observed-adverse-effect level (LOAEL) of 50 ppm for mild irritation to the eyes (6 subjects), nose (20 subjects), and throat (9 subjects). LOAEL divided by uncertainty factor of 30 (10 to protect sensitive individuals and 3 for the use of a minimal LOAEL) <sup>79</sup>
Ammonia (135 ppm)	6 subjects	5-minute exposure	Chest irritation in 1 of 6 subjects. <sup>130</sup>
Ammonia (135 ppm)	Number of subjects not available	5-minute exposure	Nose and throat irritation. <sup>130</sup>
Ammonia (135 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation. <sup>130</sup>

**Table 12. Other Clinical Reports**

Ingredient	Number of Subjects	Protocol	Results
Ammonia (25, 50, and 100 ppm)	6 subjects	Exposure: 5 days per week (2 to 6 h per day) for 6 weeks	Mild to moderate irritation of the eyes, nose and throat: 16/54 (30%) of observations on 6 subjects in week 2; 12/90 (13%) in week 3; 2/60 (3%) in week 4; 0/78 in week 5; and 5/78 (6%) in week 6. No apparent effects on pulse, respiration rate, blood pressure, FVC, or FEV <sub>1</sub> . <sup>133</sup>
Ammonia (25-100 ppm)	Not available	Exposure to varying concentrations for varying periods (2-6 h) 5 days/week for 6 weeks	Decreasing signs of irritation of the mucous membranes of the eyes, nose and throat over the 6-week observation period were reported, and there was no evidence of adverse health effects. <sup>47,133</sup>
Ammonia (72 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation. <sup>130</sup>
Ammonia (50 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation. <sup>130</sup>
Ammonia (50 ppm)	Number of subjects not available	120-minute exposure	Eye irritation. <sup>130</sup>
Ammonia (50 ppm)	Number of subjects not available	120-minute exposure	Nose and throat irritation. Urge to cough. <sup>130</sup>
Ammonia (30 and 50 ppm)	6 subjects	10-minute exposure	Barely perceptible irritant effects (nose and eye) in 2 of 6 subjects (30 ppm). Faint to moderate irritation (nose and eye) in 5 of 6 subjects (50 ppm). <sup>53</sup>
Ammonia (30 ppm and 50 ppm)	6 subjects	10-minute exposure	Moderate irritation of nose and eyes at 50 ppm (4 of 6 subjects), but not at 30 ppm. <sup>9</sup>
Ammonia (32 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation. <sup>130</sup>

## REFERENCES

1. Nikitakis, J. and Lange B. International Cosmetic Ingredient Dictionary and Handbook Online Version (wINCI). <http://webdictionary.personalcarecouncil.org/jsp/Home.jsp>. Washington, DC. Last Updated 2017. Date Accessed 3-6-2017.
2. United States Environmental Protection Agency (EPA). Toxicological review of ammonia noncancer inhalation. [https://cfpub.epa.gov/ncea/iris/iris\\_documents/documents/toxreviews/0422tr.pdf](https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0422tr.pdf). Last Updated 2016.
3. World Health Organization (WHO). Ammonia - published under the joint sponsorship of the United Nations Environment Program, the International Labor Organization, and the World Health Organization. Geneva: World Health Organization, 1986.
4. Cosmetic Ingredient Review. Safety Assessment of Phosphoric Acid and Its Salts as Used in Cosmetics. Final Report. Available for review at the Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, D.C. 20036-4702. 2016. Washington, D.C.:
5. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for ammonia (Update). *U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Division of Toxicology/Toxicology Information Branch., 1600.Clifton.Road.NE., E-29., Atlanta., GA 30333., USA, Sep.%2004.xix., 223p.I.* 2004;xix, 223p
6. European Chemicals Agency (ECHA). Registration, Evaluation, and Authorization of Chemicals (REACH) Dossier. Anhydrous Ammonia. <https://echa.europa.eu/registration-dossier/-/registered-dossier/15557>. Last Updated 2017. Date Accessed 6-8-2017.
7. Welch, A. Exposing the dangers of anhydrous ammonia. [http://journals.lww.com/tnpi/Citation/2006/11000/Exposing\\_the\\_Dangers\\_of\\_Anhydrous\\_Ammonia.8.aspx](http://journals.lww.com/tnpi/Citation/2006/11000/Exposing_the_Dangers_of_Anhydrous_Ammonia.8.aspx). Last Updated 2006. Date Accessed 5-17-2017.
8. O'Neil, M. J. The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. 15th Edition *ed.* Cambridge, UK: Royal Society of Chemistry, 2013.
9. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for ammonia. <https://www.atsdr.cdc.gov/toxprofiles/tp126.pdf>. Last Updated 2004.
10. Scifinder. Chemical Abstracts Service: Columbus, OH. CAS Registry Numbers 7664-41-7 and 1336-21-6. Substance Identifier. <http://www.cas.org/products/scifinder>. Last Updated 2017. Date Accessed 6-20-2017.
11. United States Environmental Protection Agency (EPA). Estimation Programs Interface Suite™ for Microsoft® Windows, Calculations based on KOWWIN v1.68.4.10. 2017. Washington, D.C.: EPA.
12. National Center for Biotechnology Information. Pubchem Compound Database. Compound summary for CID 14923. Ammonium Hydroxide. [https://pubchem.ncbi.nlm.nih.gov/compound/ammonium\\_hydroxide#section=Top](https://pubchem.ncbi.nlm.nih.gov/compound/ammonium_hydroxide#section=Top). Last Updated 2017. Date Accessed 10-18-2017.
13. United States Food and Drug Administration (FDA). Listing of specific substances affirmed as GRAS. Ammonium hydroxide. 21 CFR 184.1139. <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm>. Last Updated 2016. Date Accessed 6-8-2017.
14. United States Pharmacopeial Convention. Food Chemicals Codex. Tenth *ed.* Rockville, MD: The United States Pharmacopeial Convention, 2016.
15. The United States Pharmacopeial Convention. The United States Pharmacopeia (USP). Rockville, MD: The United States Pharmacopeial Convention, 2009.
16. United States Food and Drug Administration (FDA). Information supplied to FDA by industry as part of the VCRP FDA database. 2017. Washington, D.C.: FDA.
17. Personal Care Products Council. Concentration of use by FDA product category: Ammonia and Ammonium Hydroxide. Unpublished data submitted by the Personal Care Products Council on 2-2-2017. 2017.
18. European Commission. CosIng database; following Cosmetic Regulation No. 1223/2009. <http://ec.europa.eu/growth/tools-databases/cosing/>. Last Updated 2017. Date Accessed 6-8-2017.

19. Bhattacharya, S. K. Hom G. G. Fernandez C. and Hom L. G. Ocular effects of exposure to industrial chemicals: Clinical management and proteomic approaches to damage assessment. *Cutaneous and Ocular Toxicology*. 2007;26(3):203-225.
20. National Industrial Chemicals Notification and Assessment Scheme (NICNAS). Human health tier II assessment for ammonia and ammonium hydroxide. [https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-group-assessment-report?assessment\\_id=1180](https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-group-assessment-report?assessment_id=1180). Last Updated 2013. Date Accessed 6-8-2017.
21. United States Food and Drug Administration (FDA). Drugs@FDA: FDA Approved Drug Products. Ammonia. <https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=browseByLetter.page&productLetter=A>. Last Updated 2017. Date Accessed 6-11-2017.
22. United States Food and Drug Administration (FDA). Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/CFRSearch.cfm>. Last Updated 2016. Date Accessed 6-11-2017.
23. Souba, W. W. Review. Interorgan ammonia metabolism in health and disease: A surgeon's view. *Journal of Parenteral and Enteral Nutrition*. 1987;11(6):569-579.
24. Cavender, F. and Milner G. Exposure to ammonia. Salem, H. and Katz S. A. In: *Inhalation Toxicology*. 3rd ed. Boca Raton: CRC Press; 2015:257-293.
25. Cooper, A. J. L. Ammonia metabolism in normal and portacaval-shunted rats. *Advances in Experimental Medicine and Biology*. 1990;272:23-46.
26. Dasarathy, S. Mookerjee R. P. Rackayova V. Thrane V. R. Vairappan B. Ott P. and Rose C. F. Ammonia toxicity: from head to toe? *Metab.Brain Dis*. 2017;32(2):529-538.
27. Jones, E. A. Smallwood R. A. Craigie A. and Rosenoer V. M. The enterohepatic circulation of urea nitrogen. *Clin.Sci*. 1969;37:825-836.
28. Cooper, J. L. A. and Plum F. Biochemistry and physiology of brain ammonia. *Physiol.Rev*. 1987;67:440-519.
29. Brusilow, S. W. Koehler R. C. Traystman R. J. and Cooper A. J. L. Astrocyte glutamine synthetase: importance in hyperammonemic syndromes and potential target for therapy. *NeuroRx*. 2010;7:452-470.
30. Oja, S. S. Saransaari P. Korpi E. R. Neurotoxicity of ammonia. *Neurochem.Res*. 2017;42:713-720.
31. Walker, V. Ammonia metabolism and hyperammonemic disorders. *Adv.Clin.Chem*. 2014;67:73-150.
32. Odigwe, C. C. Khatiwada B. Holbrook C. Ekeh I. S. Uzoka C. Ikwu I. and Upadhyay B. Noncirrhotic hyperammonemia causing relapsing altered mental status. *Proc (Bayl.Univ.Med.Cent.)*. 2017;28(4):472-474.
33. Summerskill, V. H. J. and Wolpert E. Ammonia metabolism in the gut. *The American Journal of Clinical Nutrition*. 2017;23(5):633-639.
34. Katayama, K. Ammonia metabolism and hepatic encephalopathy. *Hepatology Research*. 2004;30S:S71-S78.
35. Vissek, W. J. Ammonia metabolism, urea cycle capacity and their biochemical assessment. *Nutrition Reviews*. 1979;37(9):273-282.
36. Sandesh, C. S. Nagamani and Erez A. A metabolic link between the urea cycle and cancer cell proliferation. DOI: 10.1080/23723556.2015.1127314. *Molecular & Cellular Oncology*. 2016;3(2):e1127314
37. Sorensen, M. Update on cerebral uptake of blood ammonia. *Metab.Brain Dis*. 2013;28:155-159.
38. Manninen, A. T. A. and Savolainen H. Effect of short-term ammonia inhalation on selected amino acids in rat brain. *Pharmacol.Toxicol*. 1989;64(3):244-246.
39. Manninen, A. Anttila S. and Savolainen H. Rat metabolic adaptation to ammonia inhalation. *Proc.Soc.Exp.Biol.Med*. 1988;187(3):278-281.
40. Schaerdel, A. D. White W. J. Lang C. M. et al. Localized and systemic effects of environmental ammonia in rats. *Lab Anim.Sci*. 1983;33(1):40-45.

41. Cooper, A. J. L. and Lai J. C. K. Cerebral ammonia metabolism in normal and hyperammonemic rats. *Neurochemical Pathology*. 1987;6:67-95.
42. Mori, S. Kaneko H. Mitsuma T. et al. Implications of gastric topical bioactive peptides in ammonia-induced acute gastric mucosal lesions in rats. *Scand.J.Gastroenterol.* 1998;33(4):386-393.
43. Ruden, C. and Hansson S. O. How accurate are the European Union's classifications of chemical substances. *Toxicology Letters*. 2003;144:159-172.
44. Organization for Economic Co-operation and Development (OECD). Final Assessment Report. SIDS Dossier on Ammonium Hydroxide. SIDS Ammonia Zip: SIDS\_Dossier\_Ammonia\_1336216.  
[http://webnet.oecd.org/HPV/UI/SIDS\\_Details.aspx?key=d5ae737b-77d7-4d61-8687-4df45f52cace&idx=0](http://webnet.oecd.org/HPV/UI/SIDS_Details.aspx?key=d5ae737b-77d7-4d61-8687-4df45f52cace&idx=0). Last Updated 2007.
45. Takeuchi, K. Ohuchi T. Harada H. et al. Irritant and protective action of urea-urease ammonia in rat gastric mucosa. *Dig.Dis.Sci.* 1995;40(2):274-281.
46. Silver, S. D. and McGrath, FP. A Comparison of Acute Toxicities of Ethylene Imine and Ammonia to Mice. *Journal of Industrial Hygiene and Toxicology*. 1948;30(1):7-9.
47. Legters, L. Biological effects of short, high-level exposure to gases: Ammonia. Contract No. DAMD17-79-C-9086. 1980. pp.1-87. Fort Detrick, Frederick, Maryland: U.S. Army Medical Research and Development Command.
48. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.
49. Barrow, C. S. Alarie Y. and Stock M. F. Sensory irritation and incapacitation evoked by thermal decomposition products of polymers and comparisons with known sensory irritants. *Arch.Environ.Health*. 1978;33:79-88.
50. Hilaldo, C. J. Casey C. J. and Furst A. Effect of ammonia on Swiss albino mice. *J.Combust.Toxicol*. 1977;4:385-388.
51. MacEwen, J. D. and Vernot, EH. Toxic Hazards Research Unit Annual Technical Report. *Aerospace Medical Research Laboratory, Air Force.Systems Command., Wright.-Patterson.Air Force.Base., Ohio., Report No.AMRL.-TR.-72.-62., NTIS AD755.-358., 162.pages., 37.references.* 1972;
52. Kapeghian, J. C. Mincer H. H. Hones A. B. et al. Acute inhalation toxicity of ammonia in mice. *Bull.Environ.Contam.Toxicol*. 1982;29:371-378.
53. MacEwen, J. D., Theodore, J, and Vernot, EH. Human Exposure to EEL Concentrations of Monomethylhydrazine. *Aerospace Medical Research Laboratory, Aerospace Division., Air Force.Systems Command., Wright.-Patterson.Air Force.Base., Ohio., Report No.AMRL.-TR.-70.-102., (Proceedings of the First Annual Conference on Environmental Toxicology, 1970).* 1970;(Proceedings of the First Annual Conference on Environmental Toxicology):355-363.
54. Pauluhn, J. Acute inhalation toxicity of ammonia: Revisiting the importance of RD50 and LCT01/50 relationships for setting emergency response guideline values. *Regulatory Toxicology and Pharmacology*. 2013;66:315-325.
55. Appelman, L. M., Ten Berge, WF, and Reuzel, PG. Acute inhalation toxicity study of ammonia in rats with variable exposure periods. *Am Ind Hyg Assoc J*. 1982;43(9):662-665.
56. Li, W. L. and Pauluhn J. Comparative assessment of sensory irritation in rats and mice nose-only exposed to dry and humidified atmospheres. *Toxicology*. 2010;276:135-142.
57. Perkins, M. W. Wong B. Tressler J. Coggins A. Rodriguez A. Devorak J. and Sciuto A. M. Assessment of inhaled ammonia-induced lung injury in rats. *Inhal.Toxicol*. 2016;28(2):71-79.
58. Perkins, M. W. Wong B. Tressler J. Rodriguez A. Sherman K. Andres J. Devorak J. Wilkins W. L. and Sciuto A. M. Adverse respiratory effects in rats following inhalation exposure to ammonia: respiratory dynamics and histopathology. *Inhalation Toxicology*. 2017;29(1):32-41.
59. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.
60. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.

61. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.
62. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.
63. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.
64. Dodd, K. T. and Gross D. R. Ammonia inhalation toxicity in cats: A study of acute and chronic respiratory dysfunction. *Arch.Environ.Health*. 1980;35:6-14.
65. Boyd, E. M., MacLachland, ML, and Perry, WF. Experimental Ammonia Gas Poisoning in Rabbits and Cats. *Journal of Industrial Hygiene and Toxicology*. 1944;26(1):29-34.
66. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. <http://webnet.oecd.org/hpv/ui/Search.aspx>. Last Updated 2007.
67. Tsujii, M. Kawano S. Tsuji S. et al. Cell kinetics of mucosal atrophy in rat stomach induced by long-term administration of ammonia. *Gastroenterology*. 1993;104(3):796-801.
68. Zissu, D. Histopathological Changes in the Respiratory Tract of Mice Exposed to Ten Families of Airborne Chemicals. *Journal of Applied Toxicology*. 1995;15(3):207-213.
69. Buckley, L. A. Jiang X. Z. James R. A. Morgan K. T. and Barrow C. S. Respiratory tract lesions induced by sensory irritants at the median respiratory rate decrease concentration. *Toxicol.Pharmacol*. 1984;74:417-429.
70. Anderson, D. P. Beard C. W. and Hanson R. P. The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. *Avian.Dis*. 1964;8:369-379.
71. Gaafar, H. Girgis R. and Hussein M. et al. The effect of ammonia on the respiratory nasal mucosa of mice. A histological and histochemical study. *Acta Otolaryngol (Stockh)*. 1992;112(2):339-342.
72. Broderson, J. R. Lindsey J. R. and Crawford J. E. The role of environmental ammonia in respiratory mycoplasmosis of rats. *Am.J.Pathol*. 1976;85:115-130.
73. Coon, R. A. Jones R. a. Jenkins L. T. Jr. and Siegel J. Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. *Toxicol.Appl.Pharmacol*. 1970;16:646-655.
74. Richard, D. Bouley G. and Boudene C. Effects of continuous inhalation of ammonia in the rat and mouse (French). In: Agency for Toxic Substances and Disease Registry (ATSDR). 2004. Toxicological profile for ammonia. <https://www.atsdr.cdc.gov/toxprofiles/tp126.pdf>. Last Updated 2004. Date Accessed 5-23-0017.
75. Stombaugh, D. P., Teague, HS, and Roller, WL. Effects of Atmospheric Ammonia on the Pig. *Journal of Animal.Science*. 1969;20:844-847.
76. Stolpe, J. and Sedlag R. Die Einzel- und Komplexwirkung von Ammoniak und Schwefelwasserstoff in der Luft auf kleine Versuchstiere (Ratten) bei unterschiedlichen Umweltbedingungen. *Ach.Exper.Vet.Med*. 1976;30:533-539.
77. Done, S. H. Chennells D. J. Gresham A. C. Williamson S. Hunt B. Taylor L. L. Bland V. et al. Clinical and pathological responses of weaned pigs to atmospheric ammonia and dust. *Vet.Rec*. 2005;157:71-80.
78. Urbain, B. and Gustin P. Prouvost J. F. and Ansay M. Quantitative assessment of aerial ammonia toxicity to the nasal mucosa by use of the nasal lavage method in pigs. *Am.J.Vet.Res*. 1994;55(9):1335-1340.
79. Verberk, M. M. Effects of ammonia in volunteers. *Int.Arch.Occup.Environ.Health*. 1977;39:73-81.
80. Occupational Safety and Health Administration (OSHA). Air contaminants. 29 CFR:1910.1000. [https://www.ecfr.gov/cgi-bin/text-idx?SID=c5407149c832a3a7892a2e80712a59ba&mc=true&node=se29.6.1910\\_11000&rgn=div8](https://www.ecfr.gov/cgi-bin/text-idx?SID=c5407149c832a3a7892a2e80712a59ba&mc=true&node=se29.6.1910_11000&rgn=div8). Last Updated 2017. Date Accessed 6-21-2017.
81. Weatherby, J. H. Chronic toxicity of ammonia fumes by inhalation. *Proc.Soc.Exp.Biol.Med*. 1952;81:300-301.

82. Dalhamn, T. and Reid I. Ciliary activity and histologic observations in the trachea after exposure to ammonia and carbon particles. Davies, C. N. In: *Inhaled particles and vapors II*. Elmsford, NY: Pergamon Publishing Company; 1967:299-306.
83. Fazekas, I. G. Experimental suprarenal hypertrophy induced by ammonia. *Endokrinologie*. 1939;21:315-337.
84. Ota, Y. Hasumura M. Okamura M. et al. Chronic toxicity and carcinogenicity of dietary administered ammonium sulfate in F344 rats. *Fd.Chem.Toxicol*. 2006;44:17-27.
85. Holness, D. L., Purdham, JT, and Nethercott, JR. Acute and Chronic Respiratory Effects of Occupational Exposure to Ammonia. *American Industrial Hygiene Association Journal*. 1989;50(12):646-650.
86. Curtis, S. E., Anderson, CR, Simon, J, Jensen, AH, Day, DL, and Kelley, KW. Effects Of Aerial Ammonia, Hydrogen Sulfide And Swine-House Dust On Rate Of Gain And Respiratory-Tract Structure In Swine. *Journal of Animal.Science*. 1975;41(3):735-739.
87. Ballal, S. G. Ali B. A. Albafr A. A. Ahmed H. O. and Al-Hasan A. Y. **Bronchial asthma in two chemical fertilizer producing factories in eastern Saudi Arabia**. *Tuberc.Lung.Dis*. 1998;2:330-335.
88. Ali, B. A. Ahmed H. O. Ballal S. G. and Albar A. A. Pulmonary function of workers exposed to ammonia: A study in Eastern Province of Saudi Arabia. *Int.J.Occup.Environ.Health*. 2001;7:19-22.
89. Lane, M. and Gardner D. K. Increase in postimplantation development of cultured mouse embryo by amino acids and induction of fetal retardation and exencephaly by ammonium ions. *J.Reprod.Fertil*. 1994;102(2):305-312.
90. Minana, M. D. Marcaida G. Grisolia S. et al. Prenatal exposure of rats to ammonia impairs NMDA receptor function and affords delayed protection against ammonia toxicity and glutamate neurotoxicity. *J.Neuropathol.Exp.Neurol*. 1995;54(5):644-650.
91. Diekman, M. A. Scheidt A. B. Sutton A. L. et al. Growth and reproductive performance, during exposure to ammonia, of gilts afflicted with pneumonia and atrophic rhinitis. *Am.J.Vet.Res*. 1993;54(12):2128-2131.
92. Yadav, J. S. and Kaushik V. K. Genotoxic effect of ammonia exposure on workers in a fertilizer factory. *Indian J.Exp.Biol*. 1997;35(5):487-492.
93. Uzvolgyi, E. and Bojan F. Possible in vivo formation of a carcinogenic substance from diethyl pyrocarbonate and ammonia. *J.Cancer Res.Clin.Oncol*. 1980;(97):205-207.
94. Toth, B. Hydrazine, methylhydrazine and methylhydrazine sulfate carcinogenesis in swiss mice. Failure of ammonium hydroxide to interfere in the development of tumors. *Int.J.Cancer*. 1972;9:109-118.
95. Uzvolgyi, E. and Bojan F. In vivo formation of a carcinogenic substance from diethyl pyrocarbonate in the presence of ammonia. *Arch.Toxicol.Suppl*. 1985;8:490-493.
96. Tsujii, M. Kawano S. Tsuji S. et al. Ammonia: A possible promoter in Helicobacter pylori related gastric carcinogenesis. *Cancer Lett*. 1992;65(1):15-18.
97. Tsujii, M. Kawano S. Tsuji S. et al. Mechanism for ammonia-induced promotion of gastric carcinogenesis in rats. *Carcinogenesis*. 1995;16(3):563-566.
98. Cagnon, L. and Braissant O. Hyperammonemia-induced toxicity for the developing central nervous system. *Brain Research Reviews*. 2007;56:183-197.
99. Albrecht, J. Mini-Review. Roles of neuroactive amino acids in ammonia neurotoxicity. *Journal of Neuroscience Research*. 1998;51:133-138.
100. Albrecht, J. Zelinska M. and Norenberg. Glutamine as a mediator of ammonia neurotoxicity: A critical appraisal. *Biochemical Pharmacology*. 2010;(doi:10.1016/j.bcp.2010.07.024)
101. Cooper, A. J. Role of glutamine in cerebral nitrogen metabolism and ammonia neurotoxicity. *Ment.Retard.Dev.Disabil.Res.Rev*. 2001;7:280-286.
102. Bosoi, C. R. Zwingmann C. Marin H. Parent-Robitaille C. Huynh J. Tremblay M. and Rose C. F. Increased brain lactate is central to the development of brain edema in rats with chronic liver disease. *J.Hepatol*. 2014;60:554-560.

103. Martinez-Hernandez, A. Bell K. P. and Norenberg. Glutamine synthetase: glial localization in brain. *Science*. 1977;195:1356-1358.
104. Hertz, L. and Zielke H. R. Astrocytic control of glutamatergic activity: astrocytes as stars of the show. *Trends Neurosci*. 2004;27:735-743.
105. Monfort, P. Montoliu C. Hermenegildo C. Munoz M. D. and Felipe V. Differential effects of acute and chronic hyperammonemia on signal transduction pathways associated with NMDA receptors. *Neurochemistry International*. 2000;37:249-253.
106. Marcaida, G. Felipe V. Hermenegildo C. Minana M. D. and Grisolia S. Acute ammonia toxicity is mediated by the NMDA type of glutamate receptors. *Federation of European Biochemical Society Letters*. 1992;296:67-68.
107. Hermenegildo, C. Marcaida G. Montoliu C. Grisolia S. Minana M. D. and Felipe V. NMDA receptor antagonists prevent acute ammonia toxicity in mice. *Neurochemical Research*. 1996;21:1237-1244.
108. Monfort, P. Kosenko E. Erceg S. Canales J. J. and Felipe V. Molecular mechanisms of acute ammonia toxicity: Role of NMDA receptors. *Neurochemistry International*. 2002;41:95-102.
109. Seiler, N. Review. Ammonia and Alzheimer's disease. *Neurochemistry International*. 2002;41:189-207.
110. Hoyer, S. Henneberg N. Knapp S. Lannert H. and Martin E. Brain glucose metabolism is controlled by amplification and desensitization of the neuronal insulin receptor. *Ann.N.Y.Acad.Sci*. 1996;777:374-379.
111. Sims, B. Powers R. E. Sabina R. L. and Theibert A. B. Elevated adenosine monophosphate deaminase activity in Alzheimer's disease brain. *Neurobiol.Aging*. 1998;19:385-391.
112. Targowski, S. P. Klucinski W. and Jaworek D. Effect of ammonia on viability and blastogenesis of bovine lymphocytes. *Veterinary Immunology and Immunopathology*. 1984;5:297-310.
113. Kosenko, E. Kaminsky Y. Kaminsky A. Valencia M. Lee L. Hermenegildo C. and Felipe V. Superoxide production and antioxidant enzymes in ammonia intoxication in rats. *Free Radic.Res*. 1997;27:637-644.
114. Murthy, C. R. Rama Rao K. V. Bai G. and Norenberg. Ammonia induced production of free radicals in primary cultures of rat astrocytes. *J.Neurosci.Res*. 2001;66:282-288.
115. Zielinska, M. Ruszkiewicz J. Hilgier W. Fresko I. and Albrecht J. Hyperammonemia increases the expression and activity of the glutamine/arginine transporter y + LAT2 in rat cerebral cortex: implications for the nitric oxide/cGMP pathway. *Neurochem.Int*. 2011;58:190-195.
116. Targowski, S. P. Klucinski W. Babiker S. et al. Effect of ammonia on in vivo and in vitro immune response. *Infect.Immun*. 1984;43(1):289-293.
117. Tepper, J. S. Weiss B. and Wood R. W. Alterations in behavior produced by inhaled ozone or ammonia. *Fundam.Appl.Toxicol*. 1985;5:1110-1118.
118. Perkins, M. A. Osborne R. and Johnson G. R. Development of an in vitro method for skin corrosion testing. *Fundamental and Applied Toxicology*. 1996;31:9-18.
119. Sekizawa, J. Yasuhara K. Suyama Y. Yamanaka S. Tobe M. and Nishimura M. A simple method for screening assessment of skin and eye irritation. *The Journal of Toxicological Sciences*. 1994;19:25-35.
120. Hamami, I. and Marks R. Structural determinants of the response of the skin to chemical irritants. *Contact Dermatitis*. 1988;18:71-75.
121. Frosch, P. J. and Kligman A. M. Rapid blister formation in human skin with ammonium hydroxide. *British Journal of Dermatology*. 1977;96:461-473.
122. Grove, G. L. Duncan S. and Kligman A. M. Effect of aging on the blistering of human skin with ammonium hydroxide. *British Journal of Dermatology*. 1982;107:393-400.
123. Goldberg, A. M. Product Safety Evaluation. In: *Alternative Methods in Toxicology*. Vol. 1. New York: Mary Ann Liebert, Inc.; 1983:



124. Grant, W. M. Toxicology of the eye. 2nd ed. Springfield, IL: Charles C. Thomas, 1974.
125. Murphy, J. C. Osterberg R. E. Seabaugh V. M. and Bierbower G. W. Ocular irritancy responses to various pHs of acids and bases with and without irrigation. *Toxicology*. 1982;23:281-291.
126. Jacobs, G. A. OECD eye irritation tests on 2 alkalis. *Journal of the American College of Toxicology*. 1992;11(6):727
127. Murakami, M. Saita H. Teramura S. Dekigai H. Asagoe K. Kusaka S. and Kita T. Gastric ammonia has a potent ulcerogenic action on the rat stomach. *Gastroenterology*. 1993;105:1710-1715.
128. Brautbar, N. Wu M. and Richter E. D. Chronic ammonia inhalation and interstitial pulmonary fibrosis: A case report and review of the literature. *Archives of Environmental Health*. 2003;58(9):592-596.
129. Kollef, M. H. Chronic ammonium hydroxide exposure. *Annals of Internal Medicine*. 1987;107(1):118
130. Michaels, R. A. Emergency planning and the acute toxic potency of inhaled ammonia. *Environmental Health Perspectives*. 1999;107(8):617-627.
131. Silverman, L. Whittenberger J. L. and Muller J. Physiological response of man to ammonia in low concentrations. *J.Ind.Hyg.Toxicol*. 1949;31(2):74-78.
132. Cole, T. J. Cotes J. E. Johnson G. R. Martin H. Reed J. W. and Saunders M. J. Ventilation, cardiac frequency and pattern of breathing during exercise in men exposed to o-chlorobenzylidene malonitrile (CS) and ammonia gas in low concentrations. *J.Exp.Physiol*. 1977;64:341-351.
133. Ferguson, W. S. Koch W. C. Webster L. B. and Gould J. R. Human physiological response and adaptation to ammonia. *J.Occup.Med*. 1977;19(5):319-326.
134. Nemer, M. Sikkeland L. I. B. Kasem M. Kristensen P. Nijem K. Bjertness E. Skare O. Bakke B. Kongerud J. and Skogstad M. Airway inflammation and ammonia exposure among female Palestinian hairdressers: a cross-sectional study. *Occup.Environ.Med*. 2015;72:428-434.
135. Doig, P. A. and Willoughby R. A. Response of swine to atmospheric ammonia and organic dust. *J.Am.Vet.Med.Assoc*. 1971;159(11):1353-1361.