Safety Assessment of Ammonia and Ammonium Hydroxide as Used in Cosmetics

Status: Draft Report for Panel Review
Release Date: August 18, 2017
Panel Date: September 11-12, 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 Interim Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst and Ivan Boyer, Ph.D., Toxicologist.

1620 L STREET, NW, SUITE 1200 ◊ WASHINGTON, DC 20036-4702 ◊ PH 202.331.0651 ◊ FAX 202.331.0088 ◊ CIRINFO@CIR-SAFETY.ORG

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: August 18, 2017
Subject: Draft Report on Ammonia and Ammonium Hydroxide

A Scientific Literature Review (SLR) on Ammonia and Ammonium Hydroxide was issued on July 7, 2017. The attached use concentration data that are (*ammoni092017data1* and *ammoni092017data2*) included in this report were received from the Council prior to issuance of the SLR.

Also included in this package for your review are the Draft Report (*ammoni092017rep*), the CIR report history (*ammoni092017hist*), Flow chart (*ammoni092017flow.docx*), Literature search strategy (*ammoni092017strat.docx*), Ingredient data profile (*ammoni092017prof*), 2017 FDA VCRP data (*ammoni092017FDA*), the CIR final report on Phosphoric Acid and Its Salts (*ammoni092017prev)*, and Comments that were received from the Council (*ammoni092017pcpc*), which have been addressed.

In addition to the safety test data on Ammonia and Ammonium Hydroxide that are included in the Draft Report, the following data (from the European Chemicals Agency (ECHA) registration dossier on Ammonia) on surrogate chemicals are included: data on "ammonium ion" (reproductive and developmental toxicity, genotoxicity, and carcinogenicity data; counterion 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); ammonium chloride (genotoxicity data [micronucleus test]); ammonium sulfate (oral carcinogenicity and chronic oral toxicity data); and diammonium phosphate (reproductive toxicity data). The Panel should determine whether these data on surrogate chemicals are relevant to this safety assessment. Furthermore, it should be noted that the Panel has issued a Final Report on the safety of Phosphoric Acid and Its Salts (Diammonium Phosphate included) in cosmetics, and that the Panel should also determine whether data on Diammonium Phosphate in this report are relevant to the current safety assessment and should be added.

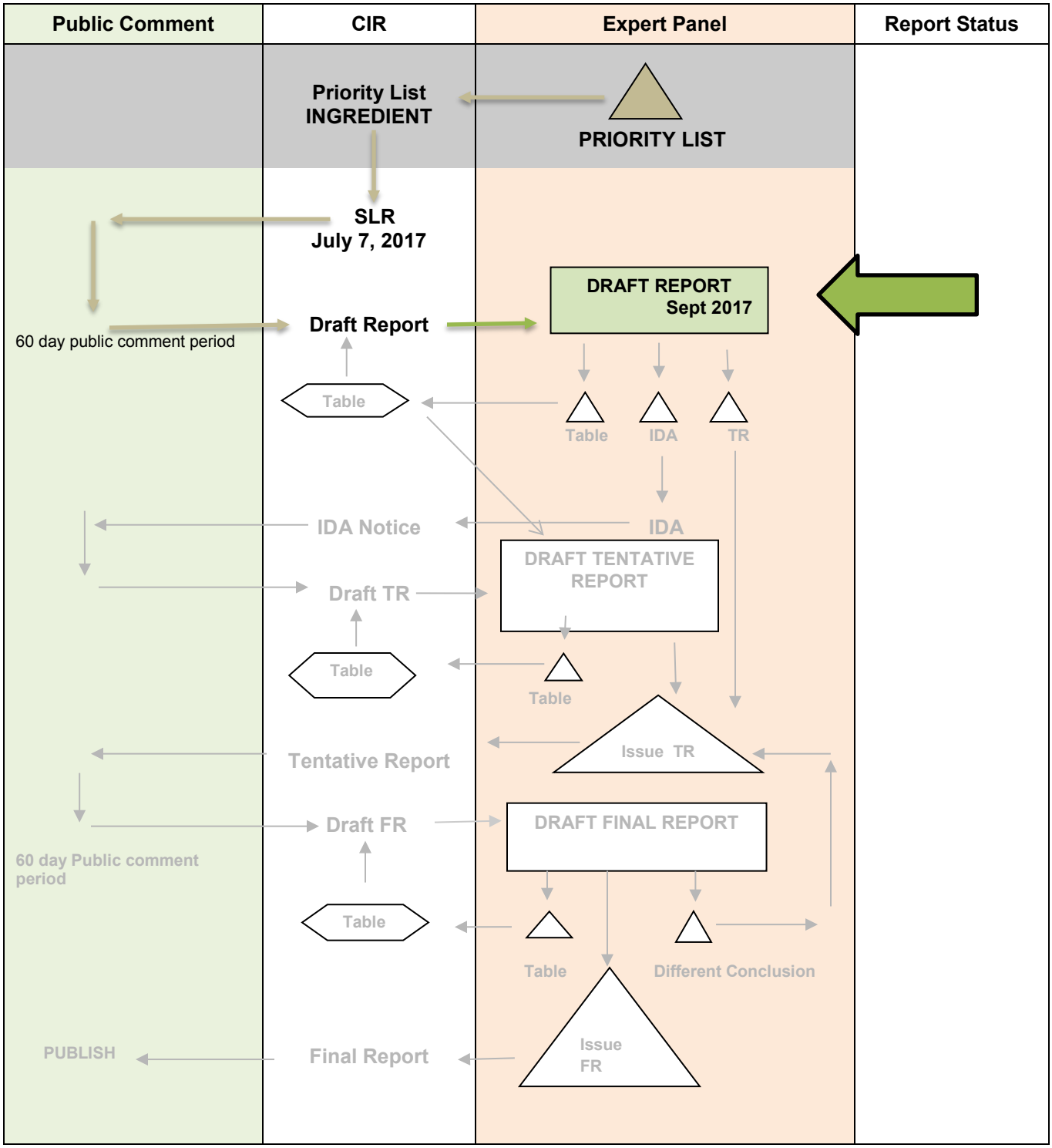
Ammonium chloride and ammonium sulfate are two of the surrogate chemicals that are mentioned above. One of the comments that was received from the Council suggests that these two cosmetic ingredients should be added to the safety assessment. However, it should be noted that the two ingredients, Ammonia and Ammonium Hydroxide, were proposed for inclusion in this safety assessment as a grouping during the priorities-setting process last year, and these additional ingredients were not recommended for inclusion. Ammonia and Ammonium Hydroxide constitute a perfect grouping because these ingredients are exactly the same thing in cosmetic products, existing with each other in equilibrium. The Panel should determine whether or not this change is warranted.

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Report with a safe as used, safe with qualifications, or unsafe conclusion. If the available data are insufficient, the Panel should issue an Insufficient Data Announcement (IDA), specifying the data needs therein.

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Ammonium Hydroxide and Ammonia

MEETING Sept 2017



CIR History of:

Ammonia and Ammonium Hydroxide

A Scientific Literature Review (SLR) on Ammonia and Ammonium Hydroxide was issued on July 7, 2017.

Draft Report, Teams/Panel: September 11-12, 2017

The draft report contains use concentration data on Ammonia and Ammonium Hydroxide that were received from the Council.

Mentha piperita-derived Ingredients Data Profile for September 11th-12th, 2017 Panel – Wilbur Johnson

			Dermal Penetration			Nail Penetration	Penetration Enhancement	ADME					Acute Toxicity			Short-Term Toxicity	Sub-Chronic Toxicity	Chronic Toxicity	DART		Genotoxicity	Carcinogenicity	Other Relevant Studies		Dermal Irritation*	Dermal Sensitization /Phototoxicity		Ocular Irritation *		Clinical Studies	Case Reports		Epidemiology Studies
	Used in Cosmetics?	Safety Data Available?	In Vivo -Animal	In Vitro-Human	In Vivo-Human	In Vitro-Human	In Vitro-Animal	In Vitro-Human Dermal	Animal-Dermal	Animal-Oral	Animal-IV	Human-Oral	Animal-Dermal	Animal-Oral	Animal-Inhalation	Animal	Animal	Animal	In Vitro	In Vivo	In Vitro/In Vivo	In Vivo	In Vitro	In Vivo-Animal	Animal/Human/In vitro	Animal	Human	In Vitro	Animal	Human-Inhalation	Human-Dermal	Human-Inhalation	Human
Ammonia	X	X								X		X		X	X	X	X		X	X	X/X	X		X	X/X			X	X	X			X
Ammonium Hydroxide	X	X																X				X			X/X/X				X				

X = data; 0 = no data

[Ammonia and Ammonium Hydroxide (3/20/2017; Updated on 8/1/2017)]

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	FEMA	ECETOC
Ammonia	7664-41-7 8007-57-6	1/1	83/563	14/455	17/283	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No
Ammonium Hydroxide	1336-21-6	1/1	20/1064	14/1159	9/366	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No

Search Strategy

[*document search strategy used for SciFinder, PubMed, and Toxnet*]

[*identify total # of hits /# hits that were useful or examined for usefulness*]

LINKS

InfoBase (self-reminder that this info has been accessed; not a public website) - http://www.personalcarecouncil.org/science-safety/line-infobase
ScfFinder (usually a combined search for all ingredients in report; list # of this/# useful) - https://scifinder.cas.org/scifinder
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - http://www.ncbi.nlm.nih.gov/pubmed
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – https://toxnet.nlm.nih.gov/ (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)

FDA databases – http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (CFR); then,
list of all databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm; then,
http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true (EAFUS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm (GRAS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm (SCOGS database);
http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives (indirect food additives list);
http://www.fda.gov/Drugs/InformationOnDrugs/default.htm (drug approvals and database);
http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf (OTC ingredient list);
http://www.accessdata.fda.gov/scripts/cder/iig/ (inactive ingredients approved for drugs)

EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - http://ec.europa.eu/growth/tools-databases/cosing/
ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/
NTIS (National Technical Information Service) - http://www.ntis.gov/
NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/ (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - http://www.ecetoc.org/

Botanical Websites, if applicable
Dr. Duke's https://phytochem.nal.usda.gov/phytochem/search
Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
Sigma Aldrich plant profiler http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html

Fragrance Websites, if applicable
IFRA (International Fragrance Association) – http://www.ifraorg.org/

RIFM (the Research Institute for Fragrance Materials) should be contacted

Safety Assessment of Ammonia and Ammonium Hydroxide as Used in Cosmetics

Status: Draft Report for Panel Review
Release Date: August 18, 2017
Panel Date: September 11-12, 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 Interim Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst and Ivan Boyer, Ph.D., Toxicologist.

1620 L STREET, NW, SUITE 1200 ◊ WASHINGTON, DC 20036-4702 ◊ PH 202.331.0651 ◊ FAX 202.331.0088 ◊ CIRINFO@CIR-SAFETY.ORG

INTRODUCTION

The safety of Ammonia and Ammonium Hydroxide in cosmetics is reviewed in this Cosmetic Ingredient Review (CIR) safety assessment. According to the *International Cosmetic Ingredient Dictionary and Handbook* (Dictionary), both ingredients are reported to function as pH adjusters in cosmetic products.[1] Additionally, Ammonia is reported to function as an external analgesic and fragrance ingredient and Ammonium Hydroxide is reported to function as adenaturant in cosmetic products. Functioning as an external analgesic is not a cosmetic use and, therefore, the CIR Expert Panel (Panel) will not evaluate safety in relation to that use in cosmetics. Additionally, the function of fragrance may be excluded from the purview of the Panel.

In 2016, the Panel issued a Final Report on Phosphoric Acid and Its Salts (Diammonium Phosphate included) with a conclusion stating that these ingredients are safe in the present practices of use and concentrations in cosmetics when formulated to be non-irritating.[2] It is possible that data on the diammonium salt, Diammonium Phosphate, may be relevant to the current safety assessment on Ammonia and Ammonium Hydroxide.

An Agency for Toxic Substances and Disease Registry (ATSDR) toxicological profile for Ammonia was published in 2004, and many of the toxicity studies summarized in that document are also included in this CIR safety assessment.[3] Pertinent information (e.g., number of animals tested and study details) that is missing from some of the study summaries in this safety assessment is being sought.

An Environmental Protection Agency (EPA) toxicological review that covers gaseous Ammonia (NH_3) and Ammonia dissolved in water (Ammonium Hydroxide, NH_4OH) was published in 2016.[4] It should be noted that portions of the EPA review are adapted from the toxicological profile for Ammonia that was developed by the 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 safety test data on Ammonia and Ammonium Hydroxide that are included in this safety assessment, the following data on surrogate chemicals are also included: 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); ammonium chloride (genotoxicity data [micronucleus test]); and ammonium sulfate (oral carcinogenicity and chronic oral toxicity data). The European Chemicals Agency (ECHA) registration dossier on Ammonia is the source of the safety test data on diammonium phosphate, ammonium chloride, and ammonium sulfate.[5] The CIR Expert Panel (Panel) will determine whether or not data on these surrogate chemicals are useful in evaluating the safety of Ammonia and Ammonium Hydroxide in cosmetic products.

Furthermore, in addition to the ASTDR 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.[6] This assessment was published under the joint sponsorship of the United Nations Environment Program, the International Labor Organization, and the World Health Organization.

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.

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.[7] Ammonia dissolved in water is referred to as aqueous ammonia, ammonia solution, and the ingredient name, Ammonium Hydroxide. In an aqueous formulation, these two ingredients will each comprise at least some of the other.

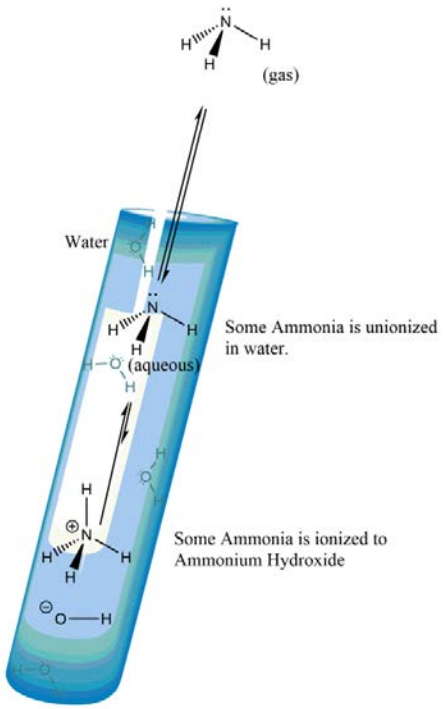


Figure 1. The aqueous relationship of Ammonia and Ammonium Hydroxide

Most inorganic hydroxides are alkaline salts formed by treating oxides with water, or via decomposing salts by adding other soluble hydroxides to a solution thereof. However, Ammonium Hydroxide is formed simply by the hydrolysis of Ammonia. Regardless of whether the ingredient is named Ammonia or Ammonium Hydroxide, if the formulation or test article is aqueous, both are present due to an equilibrium. At or near neutral pH, more than 99% is in the form of dissolved (i.e. molecular) Ammonia, and less than 1% is Ammonium Hydroxide. In more alkaline (i.e. higher pH) solutions, the Ammonium Hydroxide concentration can be significantly higher (e.g., at pH 9.25 the ratio of Ammonia to Ammonium Hydroxide is about 1:1; pK_b ~4.8 at room temperature). Accordingly, the ratio of dissolved molecular Ammonia versus the ions of Ammonium Hydroxide is dependent, *inter alia*, on the pH of the formulation. Saturation in water, at room temperature and atmospheric pressure, is approximately 34%.[8]

Application of ammonia gas (i.e., anhydrous ammonia) to cosmetics without addition to water seems unlikely, unless some other reaction product is desired. Since the functions of external analgesic and fragrance 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" refers to a ratio of hydroxide and hydronium ions in water. Accordingly, any ingredient that functions as a pH adjuster must do so in an aqueous formation. *Ipso facto*, this assessment addresses only the safety of the ingredient, Ammonia, as used in aqueous formulations. And, Ammonium Hydroxide does not exist outside of an aqueous solution. Therefore, whether Ammonia or Ammonium Hydroxide is on the cosmetic ingredient label, the chemical moieties contained therein are the same.

The definitions, structures, and functions in cosmetics of these ingredients are presented in Table 1.

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.[9] It is a weak base that exists in equilibrium with the Ammonium Hydroxide as shown in Figure 1. Ammonium Hydroxide is a salt, formed by hydrolysis of Ammonia,that essentially does not exist outside of aqueous solution.

Chemical and physical properties of Ammonia and Ammonium Hydroxide are presented in Table 2.[3,10,11]

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).[8]

Ammonium Hydroxide can be produced by passing Ammonia gas into water.[12]

Composition

According to the *Food Chemicals Codex*, Ammonium Hydroxide contains not less than 27% and not more than 30% by weight NH_3.[13] The monograph on strong Ammonia solution in the *United States Pharmacopoeia* states that this is a solution of NH_3, containing not less than 27% and not more that 31 % (w/w) NH_3.[14]

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).[13] 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).[14]

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.[15] 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.[16]

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 3).[15] Most of the uses of these 2 ingredeints are 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 that the highest maximum cosmetic use concentration of Ammonium Hydroxide is 12.5% (in rinse-off products [hair dyes and colors]) (Table 3).[16] 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 (at maximum use concentrations up to 0.58% (Ammonium Hydroxide) in eye area) and mucous membranes (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.

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 NH_3)).[17] Furthermore, the following phrase appears in the wording of "conditions of use and warnings" category: above 2%: contains Ammonia.

Noncosmetic

Ammonia is a common industrial, and naturally formed, chemical with diverse uses, such as fertilizer and as a refrigerant.[18] It is also used in production of dyes, plastics, synthetic fibers, pesticides, explosives, refrigerants, and pharmaceuticals, and in the purification of water.[7]

Ammonium Hydroxide is affirmed as generally recognized as safe (GRAS) as a direct human food ingredient.[12] This designation also means that Ammonium Hydroxide meets the specifications of the *Food Chemicals Codex* (see Impurities section).[13] Anhydrous Ammonia is used or intended for use as a source of nonprotein nitrogen in cattle feed.[19]

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.[20] 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.[21] 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.[22]

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 principle byproduct of amino acid metabolism, and the liver is indicated as the central organ of Ammonia metabolism.[9] 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.[23,24] 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.[25,26] A large amount of metabolically-generated Ammonia is absorbed into the blood and, via the portal vein, is detoxified by the liver.[25,27,28] The normal concentration of Ammonia in the portal blood varies from 300 to 600 μM. But, in the blood leaving the liver the concentration is reduced to 20–60 μM. This confirms that the liver occupies a central position in the regulation of Ammonia levels in the organism.[29,30] According to another source, the normal range for blood serum levels is of 10-40 μmol/L.[31]

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).[32] Both the gut and kidneys generate substantial amounts of Ammonia from the deamidation of glutamine.[9] The glutamine-glutamate cycle in the body works in conjunction with the glucose alanine cycle to shuttle Ammonia from peripheral to visceral organs.

Ammonia in aqueous solution (e.g., in the blood) is present as NH_3 and NH_4OH (Ammonia and Ammonium Hydroxide, respectively), with the ratio NH_3/NH_4OH depending on the pH, as defined by the Henderson-Hasselbach equation. However, contrary to expectations of simple solution phase kinetics, under physiological conditions with a blood pH of 7.4, more than 98% is in the form of NH_4OH.[25,33] Renal regulation of acid-base balance involves the formation and excretion of NH_3 to buffer hydrogen ions that are excreted in the urine. Approximately two-thirds of urinary NH_4OH is derived from the amide nitrogen of glutamine, a reaction that is catalyzed by the glutaminase enzyme in renal tubular cells.[9]

The urea cycle, a cycle of biochemical reactions that produces urea from Ammonia, is the major pathway for Ammonia detoxification in terrestrial mammals.[34] In the liver, the urea cycle is essential to the conversion of excess nitrogen from Ammonia and aspartate into urea.[35] When the supply of Ammonia in mammals exceeds the capacity for its detoxification, the excretion of orotic acid in the urine increases.[34] Orotic acid (from the urea cycle) is an intermediate product in the biosynthesis of pyrimidines.

There is evidence that Ammonia can cross blood-brain barrier (BBB), preferentially by active transport through ion transporters rather than diffusion.[25,36]

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 .[37,38]

Continuous exposure of rats for 24 h to concentrations up to 32 ppm Ammnonia resulted in significant increase in blood Ammonia levels.[39] 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 [^{13}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).[40]

Human

The first step in the degradation of most amino acids is the removal of an α-amino residue, and an amino residue is transferred to α-ketoglutaric acid to produce glutamate.[41] Glutamate dehydrogenase converts glutamate to α- ketoglutarate and Ammonia. To prevent a toxic build up 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.

TOXICOLOGICAL 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.

Acute Toxicity Studies

Acute toxicity studies (animals studies) are summarized in Table 4 (oral studies) and in Table 5 (inhalation studies).

Dermal

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

Oral

Either no effects or no serious effects were reported for Ammonia in single oral exposure animal studies. However, when 0.3% Ammonia was administered to rats by gavage (33.3 mg/kg), gastric mucosal lesions were observed within 5 minutes. An acute oral LD_{50} of 350 mg/kg for Ammonia in rats has been reported, and the oral administration of 1 % or 3% (w/w as Ammonium Hydroxide) to rats by gavage has produced severe hemorrhagic lesions.[5,42,43,44,45,46,47,48]

Inhalation

In acute inhalation toxicity studies involving mice, LC_{50}s/RD_{50}s ranging from 303 ppm to 10,150 ppm have been reported**.** In 10-minute exposure studies involving mice, LC_{50}s of $\leq$ 10,150 ppm have been reported. In mice exposed to Ammonia (100-800 ppm) for 30 minutes, an RD_{50} (exposure concentration that produced a 50% reduction in respiratory rate) of 303 ppm was reported. 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. 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. Additionally, LC_{50} values of 4837 ppm and 4230 ppm for Ammonia have been reported for 1-h exposures to 3600-5720 ppm and 1190-4860 ppm, respectively.[23,49,50,51,52,53,54,55]

In acute inahalation toxicity studies involving rats, LC_{50}s/RD_{50}s ranging from 905 ppm to 45,124 ppm have been reported. Exposure durations ranged from 10 minutes (14,170-55,289 ppm) to 1-4 h (3,028-5,053 ppm). For the 10-minute exposure, 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 LC_{50s} were ~17,633 ppm and ~7068 ppm, respectively, and corneal opacity and signs of typical upper respiratory tract irritation were observed. Signs of upper respiratory tract irritation were also associated with exposures ranging from 20 to 45 minutes, which included exposure concentrations up to 35,000 ppm. Reduced body weight was reported for rats exposed to Ammonia at a concentration of 500 ppm. No effects were observed in rats exposed to Ammonia at a concentration of 144 ppm for 5, 15, 30, or 60 minutes. Toxic signs observed in studies in which rabbits were exposed for 1 h to Ammonia at concentrations ranging from 9,800 ppm to 12,800 ppm included congestion of respiratory tract tissues, bronchiolar damage, and alveolar effects (congestion, edema, atelectasis, hemorrhage, and emphysema). At lower concentrations, there was a

significant decrease in the rate of respiration (50 ppm and 100 ppm, for 2.5-3 h) and increased respiratory tract fluid output (at 3.5 ppm and 8.7 ppm, for 1 h) in rabbits. Congestion of the respiratory tract/lungs was reported in studies in which cats were exposed to Ammonia for 1 h at concentrations ranging from 5,200 ppm to 12,800 ppm and, for 10 minutes, at a concentration of 1,000 ppm. Gross pathological findings after the 10-minute exposure included varying degrees of congestion, hemorrhage, edema, interstitial emphysema, and lung collapse.[23,5,49,56,57,58,59,60,61,62,63,64]

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.[4] Furthermore, Ammonia readily dissolves in the moisture on mucous membranes, forming Ammonium Hydroxide, which causes liquefactive necrosis of the tissues.

Short-Term Toxicity Studies

Short-term toxicity studies involving animals are summarized in Table 6 (oral and inhalation studies).

Dermal

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

Oral

Ammonia and Diammonium Phosphate (included as a potentially similar ammonium salt)

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 Ammonia (0.01% in drinking water) for 8 weeks. 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 in rats dosed orally for 5 weeks.[5,65]

Inhalation

Rats were exposed repeatedly to Ammonia at concentrations ranging from 150 ppm (for 75 days) to 1306 ppm (for 42 days (5 days/week and 8 h/day)). The higher concentration was tolerated for 42 days in rats, and increased thickness of the nasal epithelium was observed at 150 ppm. 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. 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) to 1306 ppm (42-day exposure (5 days/week and 8 h/day)) 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)) 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. In other studies, nearly 64% lethality was reported for rats exposed to Ammonia (653 ppm) for 25 days (continuous exposure) and 50 of 51 rats exposed to Ammonia (650 ppm) were dead by day 65 of continuous exposure. 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.[4,23,40, 48,56,66,67,90,68,69,70,94,95,96,71,72,73,74]

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 ppm ÷ 30 [uncertainty factor] = 1.7; uncertainty factor = 10 [to protect sensitive individuals] x 3 [for use of a minimal LOAEL] = 30).[75]

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^3) for Ammonia in the workplace.[76] Exposure to Ammonia shall not exceed the 50 ppm limit in any 8-h work shift of a 40-h work week.

Subchronic Toxicity Studies

Dermal

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

Oral

Subchronic oral toxicity data on Ammonia were not found in the published literature, nor were these data submitted.

Inhalation

Subchronic inhalation toxicity studies on Ammonia and Ammonium Hydroxide are summarized in Table 6.

Fatty changes of liver plate cells were seen in rats following continuous exposure to Ammonia (642 ppm) for 90 days. 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. Damaged tracheal mucosae were observed in rats exposed repeatedly to Ammonia (100 ppm) for 12 weeks. Mild leucocytosis 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.[49,56, 66,77,78]

A low incidence of mortalities was observed in mice and guinea pigs exposed continuously to 671 ppm Ammonia for 90 days. However, there were no mortalities in rats, guinea pigs, rabbits, monkeys, or dogs exposed continuously to ~57.43 ppm Ammonia for 114 days.[56,66]

Chronic Toxicity Studies

Dermal

Chronic dermal toxicity data on 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.[79]

Ammonium Sulfate (included as a potentially similar ammonium salt)

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 control and 3% dietary group, but 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.[5,80] Neoplastic lesions (classified as unrelated to ammonium sulfate in the diet) reported in a carcinogenicity study in the same report are included in Table 8.

Inhalation

Human

Risk Assessment

Chronic occupational exposure (about 14 years) to low levels of airborne Ammonia (12.5 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.[81] The ATSDR derived a chronic inhalation minimal risk level (MRL) of 0.1 ppm for Ammonia from this study. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of

exposure. MRLs are based on noncancer 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.[81] The cohort consisted of 52 workers and 35 controls (all males). The subjects were assessed on two workdays: on the first workday of their workweek and on the 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.[81]

Based on occupational epidemiology studies, the EPA calculated a chronic inhalation reference concentration (RfC) of 0.5 mg/m^3.[4] The critical effects in these studies were decreased lung function and respiratory symptoms.[82,81,83,84] 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.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Developmental/reproductive toxicity studies are summarized in Table 7.

Ammonia and Diammonium Phosphate (included as a potentially similar ammonium salt)

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 µmol/l) for up to 93 h. In a developmental toxicity study involving pregnant rats exposed to Ammonia in the diet (4293 mg/kg/day; as the ammonium ion) from gestation day 1 through day 21 of lactation, body weights of offspring were reduced by 25% (males) and 16% (females). 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. In a reproductive and developmental toxicity study on diammonium phosphate involving rats (oral dosing), an NOAEL of 1500 mg/kg/day and an LOAEL of >1500 mg/kg/day were reported. The only histological findings relating to maternal toxicity 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 tye result of an irritant effect.[3,5,48,56,85, 86,87]

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.[5,56,48]

Ammonia was non-genotoxic to *E. coli* strain Sd-4-73 in an in vitro assay without metabolic activation.[48]

In Vivo

Femoral bone marrow cells were examined for polychromatic erythrocytes, and there was no evidence of genotoxicity at the doses administered. 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, sister chromatid exchanges, and mitotic index, with increasing duration of exposure. However, regarding these results, it has been noted that there are a number of limitations in this study, including gaps in the analysis, small study size, and possible confounding factors such as smoking and exposure to other chemicals.[3,5,20,48,56,88]

Ammonia and Ammonium Chloride (included as a potentially similar ammonium salt)

An increased frequency of micronuclei (compared to controls) was observed in Swiss albino mice that received single intraperitoneal doses of Ammonia (12, 25, or 50 mg/kg). In the micronucleus test, groups of 10 (5 males, 5 females) ddY mice received single intraperitoneal (i.p.) doses of 62.5, 125, 250 and 500 mg/kg ammonium chloride or i.p. doses of 31.3, 62.5, 125, and 250 mg/kg ammonium chloride (4 injections within 24 h).[5]

CARCINOGENICITY STUDIES

Carcinogenicity and tumor promotion studies are summarized in Table 8.

Ammonia and Ammonium Sulfate (included as a potentially similar ammonium salt)

There was no evidence of carcinogenicity in mice dosed orally with Ammonia (dissolved in water; 42 mg /kg/day; as the ammonium ion) for 4 weeks. Following the oral dosing of mice (Swiss and C3H) with Ammonia (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). The life-time oral administration of Ammonia (in drinking water) to Swiss and C3H mice was not associated with any carcinogenic effects. 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.[5,48,56,80,89,90,91,92]

The carcinogenicity of ammonium sulfate was evaluated using groups of 100 (50 males, 50 females per group) F344 rats fed concentrations of 0% (control), 1.5%, or 3% in the diet for 104 weeks. All animals that survived were killed and subjected to a necropsy at the end of the treatment period, and the same was true for animals that died or became moribund during the treatment period as soon as they were found. Survival rates for the control, 1.5% and 3% dietary groups were 88%, 78%, and 76%, respectively, for males and 76%, 80%, and 80%, respectively, for females. With the exception of massive, nodular or focal lesions suggestive of neoplastic change, there were no obvious macroscopic findings in any of the groups tested. The following neoplastic lesions were observed: in all groups: C-cell adenomas/adenocarcinomas in the thyroids, fibroadenomas/adenomas/adenocarcinomas in the mammary glands, adenomas/adenocarcinomas in the pituitary glands, interstitial cell tumors in the testes, and endometrial stromal polyps in the uteri. However, it was noted that these neoplastic lesions are all known to occur spontaneously in F344 rats and that neither increases in the incidences nor specific types of these lesions were observed in in groups fed ammonium sulfate in the diet. Regarding non-neoplastic lesions, the incidence of chronic nephropathy in the kidney was statistically significantly increased in males of the 1.5% dietary group. Altered hepatocellular foci and bile duct proliferation in the liver and retinal atrophy in the eye were observed in control and treatment groups ; however, no statistically significant intergroup differences in the incidence or severity of these lesions was noted.

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.[93] In another study, the size, depth, and metastasis of MNNG-initiated tumors was enhanced in rats dosed orally with Ammonia (~42 mg/kg/day).[94]

OTHER RELEVANT STUDIES

Neurotoxicity

Ammonia is most toxic in the brain, and chronic hyperammonemia (metabolic disturbance) may lead to brain damage, especially in children.[9] 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.[95] 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 overactivation of ionotropic glutamate (GLU) receptors, mainly the *N*-methyl-D-aspartate (NMDA) receptors, and, to a lesser degree, the kainic acid (KA)/α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. [96] Results from other studies suggest that glutamine is also a mediator of Ammonia neurotoxicity.[97,98]

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.[25,99] 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).[100,101]

In in vitro studies, it has been demonstrated that acute intoxication with large doses of Ammonia leads to excessive activation of NMDA receptors.[102,103,104,105] 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.[102]

Brain Pathology

The excessive formation of Ammonia within the brains of Alzheimer's disease patients and its release into the periphery has been demonstrated.[106,107] 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.[106,108]

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).[109] 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% ± 8 at 24 h, 40% ± 7 at 48 h, and to 39% ± 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.[109] 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-^{3}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 ug/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 µg/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,[110,111] leading to excessive production of nitric oxide (NO) by stimulating the citrulline-NO cycle.[112]

Immunological Effects

When guinea pigs were exposed to 6.75 µg of Ammonia per deciliter of air (90 ppm), the delayed response to tuberculin injected 3 weeks later was statistically significantly less in experimental guinea pigs than in control guinea pigs. Thus, a significant decrease in the cell-mediated immune response to challenge with tuberculin was noted.[113] Furthermore, the response of blood and bronchial lymphocytes to mitogens (phytohemagglutinin, concanavalin A, purified protein derivative of tuberculin) was markedly reduced.

A delayed-type hypersensitivity test was used to evaluate cell-mediated immunity in groups of 8 Hartley guinea pigs.[113] The animals were vaccinated with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) and exposed to Ammonia (< 15 ppm, 50 ppm, or 90 ppm) for 3 weeks. Exposure to Ammonia was followed by intradermal challenge with a purified protein derivative. 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 phytohemagglutinin or concanavalin 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 guinea pigs and then treated with Ammonia, reduced proliferation and bactericidal capacity were observed only at concentrations that reduced viability. 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 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.[114]

DERMAL IRRITATION AND SENSITZATION STUDIES

Dermal irritation studies are summarized in Table 9.

Irritation

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. 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). 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.[115] Ammonia (20% as Ammonium Hydroxide) was corrosive to the skin of rabbits. In another study involving rabbits, 12% aqueous Ammonia was corrosive to the skin, whereas 10% was not. 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. 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. 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.[5,20,48,116,115,117,118,119]

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 10.

Ammonia (as Ammonium Hydroxide) at 1 mg was classified as an ocular irritant in rabbits. At a concentration of 28.5%, Ammonia induced corneal opacity in rabbits. In a study involving groups of 6 rabbits, Ammonia caused conjunctivitis at concentrations of 1% to 10%, but not 0.3%; the 10% concentration also caused corneal opacities within 1 h of instillation. Conjunctivitis and corneal damage were also observed in a study involving 3 rabbits, whereby 3% Ammonia, 100 µl was instilled into the eyes. Ammonia was classified as a severe ocular irritant in the in vitro ^{51}Cr-release assay involving human corneal endothelial cell cultures.[3,48,120,121,122,123]

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. It has been reported that Ammonia can penetrate the eye rapidly and that ocular irritation or damage can occur at concentrations as low as 20 ppm.[18,23,39]

MUCOUS MEMBRANE IRRITATION STUDIES

The stomachs of male Sprague-Dawley rats were exposed (mounted in 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.[124]

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.[125]

A male custodian had used Ammonia (28% Ammonium Hydroxide solution; which he dilutes in water) to clean office floors daily for 19 years.[126] 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 11.

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.[49,75,127,128,129,130,131]

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.[5]

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). 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 adjustor (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, via the portal vein, is detoxified by theliver. 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. Ammonia in aqueous solution (e.g., in the blood) is present as NH_3 and NH_4OH (Ammonia and Ammonium Hydroxide, respectively), with the ratio NH_3/NH_4OH depending on the pH, as defined by the Henderson-Hasselbach equation. The normal range for blood serum levels has been reported as 10-40 µmol/L.

An acute oral 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, LC_{50s} of $\leq$ 10,150 ppm have been reported. In mice exposed to Ammonia (100 - 800 ppm) for 30 minutes, an 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 (3,028-5,053 ppm) in acute inhalation toxicity studies involving rats. For the 10-minute exposure, 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 LC_{50s} 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 1,306 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 leucocytosis 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 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 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) 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 a reproductive and developmental toxicity study on diammonium phosphate involving rats, a NOAEL of 1500 mg/kg/day and an LOAEL of >1500 mg/kg/day were 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 nongenotoxic to *E. coli* strain Sd-4-73. An increased frequency of micronuclei (compared to controls) was observed in Swiss albino mice that received single intraperitoneal doses. Ammonium chloride was non-genotoxic in ddY mice the micronucleus test.

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. However, it was noted that some of the limitations associated with this study include small study size and confounding factors such as smoking and exposure to other chemicals.

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 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.

It has been reported that hyperammonemia (a metabolic disturbance characterised 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 1,105 ppm Ammonia for 6 weeks.

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%.

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.[115] 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.

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.

DISCUSSION

To be developed.

CONCLUSION

To be determined.

Table 1.Definition, Idealized Structures, and Functions of the Ingredients in this Safety Assessment.[1; CIR Staff]

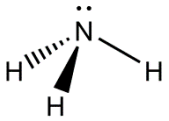
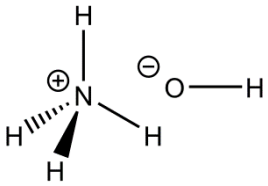
Ingredient CAS No.	Definition & Idealized Structures	Function
Ammonia	Ammonia is an inorganic gas that conforms to the formula:  (See also Ammonium Hydroxide)	External Analgesics; Fragrance Ingredients; pH Adjusters
Ammonium Hydroxide	Ammonium Hydroxide is an inorganic base that conforms to the formula:  [In reality however, the solid, anhydrous salt does not exist. Instead, Ammonium Hydroxide is only present as an aqueous ion pair, the result of hydrolysis (not dissociation of a solid salt), in equilibrium with dissolved ammonia]	Denaturants; pH Adjusters

Table 2. Physical and Chemical Properties of Ammonia and Ammonium Hydroxide

Property	Value	Reference
Ammonia		[3]
physical form and/or color	Gas at room temperature; colorless	[3]
molecular weight (Daltons (Da))	17.03	[3]
water solubility (% w/w at 20°C)	33.1	[3]
Other solubility (%w/w at 25°C)	10 (absolute ethanol); 16 (methanol); soluble in chloroform and ether	[3]
density (g/L)	0.7710 (gas);	[3]
density (g/L at -33.5°C and 1 atm)	0.6818 (liquid); 0.7 (liquid)	[3,10]
vapor density (air = 1)	0.5967	[3]
specific gravity (g/L at 25°C)	0.747	[3]
melting point (°C)	-77.7	[3,10]
boiling point (°C)	-33.35	[3,10]
autoignition temperature (°C)	650	[3]
vapor pressure (atm at 20°C)	8.5	[3]
log K_{ow} (estimated)	0.23	[3]
Ammonium Hydroxide		
density (g/L at 20°C)	0.89801(28% aqueous)	[3]
Formula weight (Da)	35.05	[10]
log K_{ow} (estimated)	-4.37	[11]

Table 3. Frequency and Concentration of Use According to Duration and Type of Exposure.[15,16]

	Ammonia		Ammonium Hydroxide		
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	
Totals/Conc. Range	599	0.00002-4.6	1354	0.00028-12.5	
Duration of Use					
Leave-On	7	0.00002-0.73	163	0.003-1.5	
Rinse off	592	0.00015-4.6	1191	0.00028-12.5	
Diluted for (bath) Use	NR	NR	NR	NR	
Exposure Type					
Eye Area	1	NR	42	0.022-0.58	
Incidental Ingestion	NR	NR	NR	NR	
Incidental Inhalation- Sprays	3***	0.73*	6*	0.29-1.3*	
Incidental Inhalation- Powders	3***	0.00002-0.14**	NR	0.45-1.5**	
Dermal Contact	6	0.00002-0.14	159	0.0012-1.7	
Deodorant (underarm)	NR	NR	NR	NR	
Hair - Non-Coloring	10	0.00006-1.4	72	0.00028-3.6	
Hair-Coloring	582	2.8-4.6	1104	2.5-12.5	
Nail	1	0.00008-0.00075	3	0.003-1.2	
Mucous Membrane	NR	NR	1	NR	
Baby Products	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 4. 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 as increase in gastric juice pH and the ulcer index in an Ammonia concentration-dependent manner (mean pH: 4.38 to 8.45).[45]
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_{50} (calculated) = 350 mg/kg.[5,46,48]
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^2 and 97.7 ± 8.3 mm^2, 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. [47]

Table 5. 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.[23,50]
Ammonia (8,770-12,940 ppm)	Mice (groups of 20). 10-minute exposure	LC_{50} =10,150 ppm.[49,51,56]
Ammonia (8,723-12,870 ppm)	Mice. 10-minute exposure	At 8,723 ppm, 25% of the animals died. At 12,870 ppm, and 80% of the animals died. LC_{50} = 10,096 ppm.[23,51]
Ammonia (3,600-5,720 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_{50} = 4837 ppm (95% CI = 4409–5305 ppm).[23,53,56]
Ammonia (1,190-4,860 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_{50} = 4,230 ppm.[23,52,56]
Ammonia (4,840 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.[23,54]
Ammonia (3,440 ppm)	Mice. 1-h exposure	Liver necrosis.[52]
Ammonia (92 mg/m^3 [~132 ppm] to 1243 mg/m^3 [~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_{50} (exposure concentration producing a 50% decrease in respiratory rate) = 582 [407 ppm] and 732 mg/m^3 [547 ppm] in dry and wet air, respectively).[23,61]
Ammonia (100-800 ppm)	Male Swiss-Webster mice. 30-minute exposure	RD_{50} = 303 ppm (95% confidence limits = 188–490 ppm).[23,55,56]
Ammonia (9,870 mg/m^3 [14,170 ppm] to 37,820 mg/m^3 [54,289 ppm])	SPF-bred Wistar rats (5 males, 5 females/group). 10-minute exposure to 54, 289 ppm and 60-minute exposure to 14,170 ppm	LC_{50} (higher concentration) = 15,940 mg/m^3 (~22,885 ppm) (males) and 31,430 mg/m^3 (~45,124 ppm) (females). LC_{50} (lower concentration) = 9,850 mg/m^3 (~14,141 ppm) (males) and 13,770 mg/m^3 (~19,769 ppm) (females). Hemorrhagic lungs in animals that died.[5,57]
Ammonia (9,000-35,000 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-out 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.[58]

Table 5. Acute Inhalation Toxicity

Ingredient	Animals/Protocol	Results
Ammonia (9,000 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.[59]
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^3 [~17,633 ppm]). LC_{50} (4-h exposure = 4,923 mg/m^3 [~7068 ppm]).[60] -
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).[23,53,56]
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.[56]
Ammonia (1436, 4307, and 6814 ppm)	White rats. Inhalation exposure for 5, 15, 30, or 60 minutes	Dyspnea, irritation of respiratory tract and eyes, cyanosis of extremities, and increased excitability.[56]
Ammonia (92 mg/m^3 [~132 ppm] to 1243 mg/m^3 [~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^3 (corresponding to ~1396 and ~1299 ppm, respectively) in rats in dry and wet air, respectively.[23,61]
Ammonia (500 ppm)	Rats. Continuous inhalation exposure for up to 8 weeks	After 3 weeks of exposure, nasal irritation and inflammation of upper respiratory tract. Signs had cleared by week 8.[62]
Ammonia (144 ppm)	Rats. Inhalation exposure for 5, 10, 15, 30, or 60 minutes	No effects.[56]
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.[23]
Ammonia (10,360 ppm, average)	Rabbits. 1-h exposure	Congestion of respiratory tract tissues.[23]
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.[56]

Table 5. Acute Inhalation Toxicity

Ingredient	Animals/Protocol	Results
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.[56]
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.[49,63]
Ammonia (10,360 ppm, average)	Cats. 1-h exposure	Congestion of respiratory tract tissues.[49,63] –
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.[56,64]
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.[56]

Table 6. Short-Term and Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
Short-term Oral Studies			
Ammonia (0.01% in drinking water)	Rats	~ 42 mg/kg/day for 8 weeks	Mucosal atrophy in stomach antrum and enlargement of proliferative zone in antral and body mucosa. [65]
diammonium phosphate (17.9% NH_4 and 46.86% P_2O_5 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. LOAEL for general toxicity = 750 mg/kg/day.[5,56,48]
Short-term Inhalation Studies			
Ammonia (~1,306 ppm)	Rats	5 days/week (8 h/day)	Exposure tolerated for 42 days. [66]
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 (Saimiri sciureus, 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[4,40,66]
Ammonia (1,086 ppm)	Rats, squirrel monkeys, and guinea pigs	Inhalation exposure 5 days per week (8 h/day) for 6 weeks	No fatty changes of liver plate cells. No pathological changes in kidney.[66]
Ammonia (653 ppm)	Rats	Continuous inhalation exposure for 25 days	Nearly 64% lethality.[66]
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.[4,66]
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).[49,66]
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.[56,62]

Table 6. Short-Term and Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
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.[4,67]
Ammonia (221 ppm; Ct [ppm-h] = 53,040)	Rats, guinea pigs, rabbits, squirrel monkeys, and beagle dogs	5 days per week (8 h per day) for 6 weeks	No effect.[49,66]
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.[4,56,67]
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.[56,73]
Ammonia (12% solution)	50 maleWhite 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 *in situ* in one nostril. At week 8, one mouse had an invasive adenocarcinoma of the nasal mucosa. Histo-chemical results were also abnormal.[4,92]
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.[23,68]
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.[23,69]
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.[4,70]
Ammonia (170 ppm; Ct [ppm-h] =30,600 to 91,800)	Guinea pigs	5 days per week (6 h per day) for 6 weeks	No histopathologic changes.[49,77]

Table 6. Short-Term and Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
Ammonia (50 ppm)	Guinea pigs (males and females, groups of 6)	Exposure for 42 days	Lung congestion, edema, and hemorrhage.[4,70]
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. [4,70]
Ammonia (100 ppm [average range = 20 to 203 ppm; Ct [ppm-h] =100,800) alone and with con 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.[4, 49,132]
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.[5,49,74]
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.[4,74]
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.[71]
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.[4, 72]
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.[4, 72]
Subchronic Inhalation Studies			
Ammonia (642 ppm)	Rats	Continuous exposure for 90 days	Fatty changes of liver plate cells.[66]
Ammonia (43 ppm or 143 ppm)	White rats	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.[56]
Ammonia (100 ppm)	Rats	Inhalation exposure 5 days per week (5 h/day) for 12 weeks	Damaged tracheal mucosae.

Table 6. 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
Ammonium Hydroxide (671 ppm)	515 rats and 15 guinea pigs	Inhalation exposure continuously for 90 days	13 rats and 4 guinea pigs died.[56]
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.[66]

Table 7. Developmental and Reproductive Toxicity Studies

Ingredient	Animals/Embryos	Protocol	Results
In Vitro Study			
Ammonium ion (38 to 300 µmol/l)	Mouse embryos (conceived in vivo)	Embryos cultured in modified mouse tubal fluid medium (mMTF) or mMTF supplemented with 300 µmol/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 µmol/L) and decreased percentage of implantation sites with increased ammonium concentration.[86]
Oral Studies			
ammonium ion	Pregnant rats	Feeding with ammonium ion in the diet (4293 mg ammonium/kg/day) from gestation day 1 through day 21 of lactation	Body weights of offspring reduced by 25% (males) and 16% (females).[3,87]
diammonium phosphate (17.9% NH_4 and 46.86% P_2O_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 = > 1500 mg/kg/day.[5,48]
diammonium phosphate	Groups of 10 (5 males, 5 females) Crj: CD(SD) rats	Administered by gavage daily for, at most, 28 days (males) and 53 days (females). Doses of 0, 250, 750, and 1500 mg/kg/day.	Mating performance and fertility unaffected by dosing. Also, dosing had no apparent effect on offspring up to 4 days of age. NOAEL (for reproductive and developmental toxicity) = 1500 mg/kg/day; LOAEL = 1500 mg/kg/day.[5,56]
Inhalation Study			
Ammonia (7 ppm or 35 ppm)	Female pigs	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.[85]

Table 8. 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.	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.[89]
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.[48, 56,90]
Ammonium ion (and diethyl pyrocarbonate)	Pregnant mice	Exposure (by gavage) during pregnancy and lactation	No lung tumors.[91]
Ammonium Sulfate	Groups of 100 F344/DuCrj rats (50 males and 50females per group)	Dietary concentrations of 0%, 1.5%, and 3% daily for 104 weeks	Survival rates of control, 1.5%, and 3% groups were 88%, 78%, and 76%, respectively, for males, and 76%, 80%, and 80%, respectively, for females. The only macroscopic findings at necropsy were massive, nodular or focal lesions suggestive of neoplastic change. The following neoplastic lesions (not treatment-related; occur spontaneously in rats of this strain) were observed: C-cell adenomas/adenocarcinomas in the thyroids, fibroadenomas/adenomas/adeno-carcinomas in mammary glands, adenomas/adenocarcinomas in pituitary glands, interstitial cell tumors in testes, and endometrial stromal polyps in uteri. Regarding non-neoplastic lesions, incidence of chronic nephropathy satistically significantly increased in males of 1.5% dietary group. No evidence of long-term carcinogenic activity.[80]
Ammonium Sulfate	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.[5]

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 from (weeks 4 to 8) from crowding of cells forming crypts and irregular arrangements to epithelial hyperplasia, patches of squamous metaplasia, loss of cilia, and dysplasia of the nasal epithelium. One mouse had a carcinoma *in situ* 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.[92]
Tumor Promotion			
Ammonia (dissolved in water)	Rats	Rats pretreated with the initiator *N*-methyl-*N'*-nitro-*N*-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).[56,93]
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.[94]

Table 9. Dermal Irritation Studies

Ingredient	Animals/Subjects/Cells	Protocol	Results
Skin Irritation Studies			
In Vitro Studies			
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., t50 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 (t50 = 0.90 minutes).[116]
Animal Studies			
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.[115]
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).[115]
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% concentratyion.[20,48]
Ammonium Hydroxide (10% and 12% aqueous)	Female Albino New Zealand White rabbits	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.[5]
Human Studies			
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.[117]

Table 9. Dermal Irritation Studies

Ingredient	Animals/Subjects/Cells	Protocol	Results
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.[118]
Ammonium Hydroxide solution (50% solution)	Young adults and older adults	Acrylic plastic block with 14 mm well loosely strapped to skin. Well was then filled with 0.5 ml of freshly pepared 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.[119]

Table 10. Ocular Irritation Studies

Ingredient	Animals/Cells	Test Protocol	Results
In Vitro Ammonium Hydroxide	Human corneal endothelial cell cultures	^{51}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_{50} = 3.9 x 10^{-3} M).[120]
Animal			
Ammonia	Not available	Not available	Ammonia can penetrate the eye rapidly. Ocular irritation or damage can occur at concentrations beginning at 20 ppm.[18]
Ammonia (15, 32, 310, or 1157 ppm vapor concentrations)	Rats	Exposure for 24 h	No clinical signs or evidence of irritation to the eyes or mucous membranes.[23,39]
Ammonium Hydroxide	Rabbits	Instillation of test substance (1 mg) followed by ocular rinsing	Ocular irritant.[48]
Ammonium Hydroxide (28.5%)	Rabbits	Brief exposures (2 seconds)	Corneal opacity.[3,121]
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.[122]
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.[123]

Table 11. Other Clinical Reports

Ingredient	Number of Subjects	Protocol	Results
Inhalation Exposure			
Ammonia (700 ppm)	Number of subjects not available	Not available	Eye irritation.[127]
Ammonia (500 ppm)	Number of subjects not available	30-minute exposure	Variable lacrimation.[127]
Ammonia (500 ppm)	Number of subjects not available	30-minute exposure	Increased blood pressure and pulse rate.[127]
Ammonia (500 pm)	Number of subjects not available	30-minute exposure	Nasal and throat irritation, increased minute volume, and cyclic pattern of hyperpnea.[127]
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.[128]
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.[49,128]
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.[49,129]
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_1), or forced inspiratory volume inhaled at end of 1st second of forced inspiration (FIV_1). 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)[75]
Ammonia (135 ppm)	6 subjects	5-minute exposure	Chest irritation in 1 of 6 subjects.[127]
Ammonia (135 ppm)	Number of subjects not available	5-minute exposure	Nose and throat irritation.[127]
Ammonia (135 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation.[127]

Table 11. 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_1.[130]
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.[49,130]
Ammonia (72 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation.[127]
Ammonia (50 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation.[127]
Ammonia (50 ppm)	Number of subjects not available	120-minute exposure	Eye irritation.[127]
Ammonia (50 ppm)	Number of subjects not available	120-minute exposure	Nose and throat irritation. Urge to cough.[127]
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).[54]
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.[54]
Ammonia (32 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation.[127]
Ammonia (> 30 ppm)	Not available	Not available	Immediate irritation of the nose and throat.[54,131,75]
Ammonia	Not available	Not available	Tolerance appears to develop with repeated exposure.[131,75]

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. 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. Washiington, D.C.:

3. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for ammonia. https://www.atsdr.cdc.gov/toxprofiles/tp126.pdf. Last Updated 2004.

4. United States Environmental Protection Agency (EPA). Toxicological review of ammonia noncancer inhalation. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0422tr.pdf. Last Updated 2016.

5. 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.

6. 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.

7. Welch, A. Exposing the dangers of anhydrous ammonia. http://journals.lww.com/tnpj/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. 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.

10. Scifinder. Chemical Abstracts Service: Columbus, OH. CAS Regsitry 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. 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.

13. United States Pharmacopeial Convention. Food Chemicals Codex. Tenth *ed*. Rockville, MD: The United States Pharmacopeial Convention, 2016.

14. The United States Pharmacopoeial Convention. The United States Pharmacopeia (USP). Rockville, MD: The United States Pharmacopeial Conventioni, 2009.

15. 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.

16. 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.

17. 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.

18. 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.

19. United States Food and Drug Administration (FDA). Food additives permitted in feed and drinking water of animals. Anhydrous ammonia. 21 CFR 573.180. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm. Last Updated 2016. Date Accessed 6-8-2017.

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. 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/cfcfr/CFRSearch.cfm. Last Updated 2016. Date Accessed 6-11-2017.

23. 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.

24. Cooper, A. J. L. Ammonia metabolism in normal and portacaval-shunted rats. *Advances in Experimental Medicine and Biology*. 1990;272:23-46.

25. 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.

26. Jones, E. A. Smallwood R. A. Craigie A. and Rosenoer V. M. The enterohepatic circulation of urea nitrogen. *Clin.Sci.* 1969;37:825-836.

27. Cooper, J. L. A. and Plum F. Biochemistry and physiology of brain ammonia. *Physiol.Rev.* 1987;67:440-519.

28. 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.

29. Oja, S. S. Saransaari P. Korpi E. R. Neurotoxicity of ammonia. *Neurochem.Res.* 2017;42:713-720.

30. Walker, V. Ammonia metabolism and hyperammonemic disorders. *Adv.Clin.Chem.* 2014;67:73-150.

31. 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.

32. Summerskill, V. H. J. and Wolpert E. Ammonia metabolism in the gut. *The American Journal of Clinical Nutrition*. 2017;23(5):633-639.

33. Bromberg, P. A. Robin E. D. and Forkner C. E. J. The existence of ammonia in blood in vivo with observations on the significance of the NH_4plus minus NH_3 system. *J.Clin.Invest.* 1960;39:332-341.

34. Visek, W. J. Ammonia metabolism, urea cycle capacity and their biochemical assessment. *Nutrition Reviews*. 1979;37(9):273-282.

35. 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

36. Sorensen, M. Update on cerebral uptake of blood ammonia. *Metab.Brain Dis.* 2013;28:155-159.

37. 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.

38. Manninen, A. Anttila S. and Savolainen H. Rat metabolic adaptation to ammonia inhalation. *Proc.Soc.Exp.Biol.Med.* 1988;187(3):278-281.

39. 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.

40. Cooper, A. J. L. and Lai J. C. K. Cerebral ammonia metabolism in normal and hyperammonemic rats. *Neurochemical Pathology*. 1987;6:67-95.

41. Katayama, K. Ammonia metabolism and hepatic encephalopathy. *Hepatology Research*. 2004;30S:S71-S78.

42. Benyajati, S. and Goldstein L. Renal glutaminase adaptation and ammonia excretion in infant rats. *Am.J.Physiol.* 1975;228:693-698.

43. Koenig, H. and Koenig R. Production of acute pulmonary edema by ammonium salts. *Proc.Soc.Exp.Biol.Med.* 1949;70(3):375-380.

44. Boyd, E. M. and Seymour K. G. W. Ethylenediamine dihydrochloride or chlor-ethamine. II. Untoward and toxic reactions. *Exp.Med.Surg.* 1946;4:223-227.

45. 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.

46. Ruden, C. and Hansson S. O. How accurate are the European Union's classifications of chemical substances. *Toxicology Letters*. 2003;144:159-172.

47. 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.

48. 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. Last Updated 2007.

49. 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.

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. 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.

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. 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;

54. 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.

55. 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.

56. 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.

57. 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.

58. 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.

59. 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.

60. 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.

61. 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.

62. 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.

63. 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)

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. 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.

66. 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.

67. 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.

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. 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.

72. 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.

73. 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.

74. Stombaugh, D. P., Teague, HS, and Roller, WL. Effects of Atmospheric Ammonia on the Pig. *Journal of Animal.Science*. 1969;20:844-847.

75. Verberk, M. M. Effects of ammonia in volunteers. *Int.Arch.Occup.Environ.Health*. 1977;39:73-81.

76. 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. Last Updated 2017. Date Accessed 6-21-2017.

77. Weatherby, J. H. Chronic toxicity of ammonia fumes by inhalation. *Proc.Soc.Exp.Biol.Med.* 1952;81:300-301.

78. 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.

79. Fazekas, I. G. Experimental suprarenal hypertrophy induced by ammonia. *Endokrinologie*. 1939;21:315-337.

80. Ota, Y. Hasumura M. Okamura M. et al. Chronic toxicity and carcinogenicity of dietary adminstered ammonium sulfate in F344 rats. *Fd.Chem.Toxicol.* 2006;44:17-27.

81. 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.

82. 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.

83. 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.

84. 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.

85. 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.

86. 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.

87. 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.

88. 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.

89. 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.

90. 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.

91. 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.

92. 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.

93. 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.

94. Tsujii, M. Kawano S. Tsuji S. et al. Mechanism for ammonia-induced promotion of gastric carcinogenesis in rats. *Carcinogenesis*. 1995;16(3):563-566.

95. Cagnon, L. and Braissant O. Hyperammonemia-induced toxicity for the developing central nervous system. *Brain Research Reviews.* 2007;56:183-197.

96. Albrecht, J. Mini-Review. Roles of neuroactive amino acids in ammonia neurotoxicity. *Journal of Neuroscience Research*. 1998;51:133-138.

97. 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)

98. Cooper, A. J. Role of glutamine in cerebral nitrogen metabolism and ammonia neurotoxicity. *Ment.Retard.Dev.Disabil.Res.Rev.* 2001;7:280-286.

99. 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.

100. Martinez-Hernandez, A. Bell K. P. and Norenberg. Glutamine synthetase: glial localization in brain. *Science*. 1977;195:1356-1358.

101. Hertz, L. and Zielke H. R. Astrocytic control of glutamatergic activity: astrocytes as stars of the show. *Trends Neurosci.* 2004;27:735-743.

102. Monfort, P. Montoliu C. Hermenegildo C. Munoz M. D. and Felipo V. Differential effects of acute and chronic hyperammonemia on signal transduction pathways associated with NMDA receptors. *Neurochemistry International*. 2000;37:249-253.

103. Marcaida, G. Felipo 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.

104. Hermenegildo, C. Marcaida G. Montoliu C. Grisolia S. Minana M. D. and Felipo V. NMDA receptor antagonists prevent acute ammonia toxicity in mice. *Neurochemical Research*. 1996;21:1237-1244.

105. Monfort, P. Kosenko E. Erceg S. Canales J. J. and Felipo V. Molecular mechanisms of acute ammonia toxicity: Role of NMDA receptors. *Neurochemistry International*. 2002;41:95-102.

106. Seiler, N. Review. Ammonia and Alzheimer's disease. *Neurochemistry International*. 2002;41:189-207.

107. 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.

108. 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.

109. 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.

110. Kosenko, E. Kaminsky Y. Kaminsky A. Valencia M. Lee L. Hermenegildo C. and Felipo V. Superoxide production and antioxidant enzymes in ammonia intoxication in rats. *Free Radic.Res.* 1997;27:637-644.

111. 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.

112. 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.

113. 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.

114. 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.

115. Sekizawa, J. Yasuhara K. Suyama Y. Yamanaka S. Tobe M. and Nishimura M. A simple method for screening assessment nof skin and eye irritation. *The Journal of Toxicological Sciences*. 1994;19:25-35.

116. 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.

117. Hamami, I. and Marks R. Structural determinants of the response of the skin to chmcial irritants. *Contact Dermatitis*. 1988;18:71-75.

118. Frosch, P. J. and Kligman A. M. Rapid blister formation in human skin with ammonium hydroxide. *British Journal of Dermatology*. 1977;96:461-473.

119. 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.

120. Goldberg, A. M. Product Safety Evaluation. In: *Alternative Methods in Toxicology*. Vol. 1. New York: Mary Ann Liebert, Inc.; 1983:

121. Grant, W. M. Toxicology of the eye. 2nd *ed*. Springfield, IL: Charles C. Thomas, 1974.

122. Murphy, J. C. Osterberg R. E. Seabaugh V. M. and Bierbower G. W. Ocular irritancy responses to varous pHs of acids and bases wih and without irrigation. *Toxicology*. 1982;23:281-291.

123. Jacobs, G. A. OECD eye irritation tests on 2 alkalis. *Journal of the American College of Toxicology*. 1992;11(6):727

124. 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.

125. Brautbar, N. Wu M. and Richter E. D. Chronic ammonia inhalation and intersittial pulmonary fibrosis: A case report and review of the literature. *Archives of Environmental Health*. 2003;58(9):592-596.

126. Kollef, M. H. Chronic ammonium hydroxide exposure. *Annals of Internal Medicine*. 1987;107(1):118

127. Michaels, R. A. Emergency planning and the acute toxic potency of inhaled ammonia. *Environmental Health Perspectives*. 1999;107(8):617-627.

128. 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.

129. 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-chlorobenzylidine malonitrile (CS) and ammonia gas in low concentrations. *J.Exp.Physiol.* 1977;64:341-351.

130. 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.

131. Sekizawa, S. I. and Tsubone H. Nasal receptors responding to noxious chemical irritants. *Respir.Physiol.* 1994;96(1):37-48.

132. 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.

Safety Assessment of Phosphoric Acid and Its Salts as Used in Cosmetics

Status: Final Report
Release Date: November 16, 2016
Panel Date: September 26-27, 2016

The 2016 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 Director is Lillian J. Gill, D.P.A. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst, Ivan Boyer, Ph.D., Toxicologist, and Bart Heldreth, Ph.D., Chemist.

1620 L STREET, NW, SUITE 1200 ◊ WASHINGTON, DC 20036-4702 ◊ PH 202.331.0651 ◊ FAX 202.331.0088 ◊ CIRINFO@CIR-SAFETY.ORG

ABSTRACT: The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) reviewed the safety of Phosphoric Acid and its salts (31 ingredients), which function as buffering agents, corrosion inhibitors, chelating agents, and pH adjusters in cosmetic products. The Panel reviewed data relating to the safety of these ingredients, and concluded that Phosphoric Acid and its salts are safe in the present practices of use and concentration in cosmetics when formulated to be non-irritating.

INTRODUCTION

The safety of the following 31 ingredients, as used in cosmetics (with systematic nomenclature in parenthesis when different from the ingredient name), is reviewed in this safety assessment:

Phosphoric Acid
- (orthophosphoric acid)

Ammonium Phosphate
- (ammonium dihydrogen orthophosphate)

Dicalcium Phosphate
- (calcium hydrogen orthophosphate)

Calcium Dihydrogen Phosphate
- (calcium dihydrogen orthophosphate)

Calcium Phosphate

Calcium Potassium Sodium Phosphate
- (dicalcium potassium sodium orthophosphate)

Calcium Pyrophosphate
- (dicalcium pyrophosphate)

Diammonium Phosphate
- (ammonium hydrogen orthophosphate)

Dicalcium Phosphate Dihydrate
- (calcium hydrogen orthophosphate dihydrate)

Dipotassium Phosphate
- (dipotassium hydrogen orthophosphate)

Disodium Phosphate
- (disodium hydrogen orthophosphate)

Disodium Pyrophosphate
- (disodium dihydrogen pyrophosphate)

Magnesium Hydrogen Phosphate
- (magnesium hydrogen orthophosphate trihydrate)

Magnesium Phosphate

Metaphosphoric Acid

Pentapotassium Triphosphate
- (pentapotassium orthophosphate)

Pentasodium Triphosphate
- (pentasodium metaphosphate)

Phosphate Buffered Saline

Potassium Metaphosphate

Potassium Phosphate

Potassium Polyphosphate

Sodium Hexametaphosphate

Sodium Metaphosphate

Sodium Polyphosphate

Sodium Phosphate
- (sodium orthophosphate)

Sodium Trimetaphosphate

Tetrapotassium Pyrophosphate

Tetrasodium Pyrophosphate

Tricalcium Phosphate
- (Tricalcium orthophosphate)

Trimagnesium Phosphate
- (trimagnesium orthophosphate)

Trisodium Phosphate
- (trisodium orthophosphate)

According to the *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), the functions of these ingredients in cosmetic products include buffering agents, corrosion inhibitors, chelating agents, and pH adjusters.[1]

Three of the phosphate salt ingredients included in this safety assessment, i.e. Sodium Metaphosphate, Sodium Trimetaphosphate, and Sodium Hexametaphosphate, have been previously reviewed by the CIR Panel.[2] In 2001, the Panel concluded that these ingredients are safe for use in cosmetics when formulated to avoid skin irritation.

CHEMISTRY

Definition and Structure

The definitions, structures, and functions in cosmetics of Phosphoric Acid and its salts are presented in Table 1.

Phosphoric Acid and its salts all have the same phosphate core. Except for Phosphoric Acid and Metaphosphoric Acid, the ingredients in this report are either alkaline earth metal (Periodic Table column I or II) salts or ammonium salts of a phosphoric acid. These ingredients are related to each other as inorganic phosphates, with varying cation identity and degree of protonation. This group comprises phosphate salts for which property differences are attributable primarily to having different cation(s). Characterizing these differences in one report that addresses all of these ingredients is more informative than attempting to assess the safety of these salts in separate reports that each addresses only one ingredient.

Phosphoric Acid is a polyprotic acid which is deprotonated to mono-, di-, and tri-phosphates with rising pH.

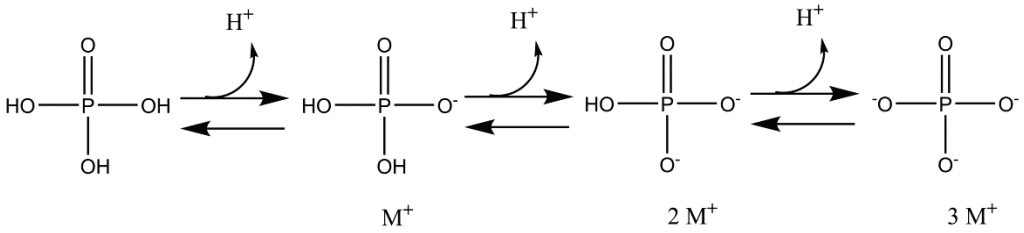


Figure1. Phosphoric Acid and the *ortho*-phosphates (dihydrogen phosphate, hydrogen phosphate, and phosphate)

However, Phosphoric Acid and phosphate salts also exist as dimers and trimers of phosphate, *pyro*- and *meta*- respectively. Accordingly, these ingredients vary by the identity of associated cations, degree of protonation, ***and*** in the number of phosphate repeat units (i.e., 1 repeat is *ortho*-, 2 repeats is *pyro*-, and 3 repeats is *meta*-).

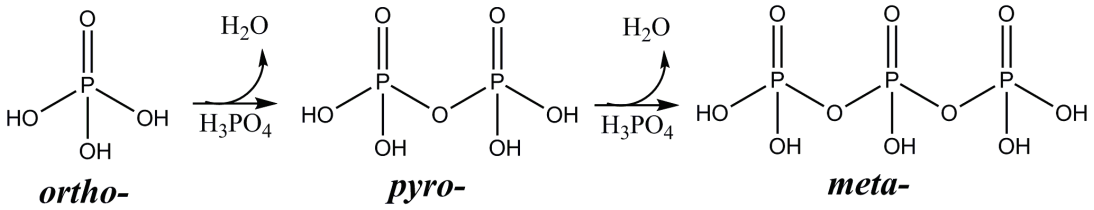


Figure 2. Dehydration of phosphoric acids, from *ortho*- to *pyro*- to *meta*-phosphoric acid.

As some of the *Dictionary* names for these ingredients vary from the customary names and may be confusing, systematic names have been, where appropriate, added to Table 1. However, elsewhere in this report only the *Dictionary* ingredient name is used.

Chemical and Physical Properties

These ingredients range from colorless crystalline solids to white amorphous powders, the water solubility of which are pH-dependent (Table 2).

Method of Manufacture

Acids

Phosphoric Acid

Phosphoric Acid is manufactured by the wet process or the furnace (thermal) process. In the wet process, Phosphoric Acid is produced directly from phosphate ores and is said to be of low purity.[3] This process is used mostly for the production of fertilizers. In the thermal or furnace process, phosphoric acid is produced from elemental phosphorus. This process is used in the production of phosphoric acid for uses other than fertilizer production, such as metal treatment, refractories, catalysts, and use in food and beverages.

Ammonium Salts

Ammonium Phosphate

In the process for manufacturing Ammonium Phosphate, a one-to-one ratio of ammonia (NH_3) and Phosphoric Acid (H_3PO_4) is reacted, and the resulting slurry of Ammonium Phosphate is solidified in a granulator.[4]

Diammonium Phosphate

In the manufacture of Diammonium Phosphate, each stoichiometric equivalent of Phosphoric Acid is neutralized by approximately 2 equivalents of ammonia.[5]

Sodium Salts

Disodium Phosphate

Disodium Phosphate is prepared by the ignition of Dicalcium Phosphate.[6]

Sodium Metaphosphate

Sodium Metaphosphate is prepared by dehydration of sodium orthophosphates.[6]

Sodium Polyphosphate

Sodium phosphate monobasic hydrate was used to prepare Sodium Polyphosphate with a degree of polymerization (D_p) lower than ≈ 500.[7] Sodium phosphate monobasic hydrate was heated to 700˚C for 1, 3, or 9 h, and the melt was then quenched on a copper plate. To fraction the Sodium Polyphosphate glass, the frit was ground and dissolved in deionized water to yield a 10% (w/v) Sodium Polyphosphate solution. The solution was stirred, fractioned by serial dilution with acetone, and then centrifuged to collect the precipitate. Sodium Polyphosphate with a $D_p > 500$ was obtained from an ion-exchange process on a potassium polyphosphate crystalline phase.

Tetrasodium Pyrophosphate

Tetrasodium Pyrophosphate is produced by molecular dehydration of dibasic Sodium Phosphate at 500˚C.[6]

Pentasodium Triphosphate

Pentasodium Triphosphate is prepared by the molecular dehydration of mono- and di-sodium phosphates.[6]

Potassium Salts

Potassium Metaphosphate

Potassium Metaphosphate is obtained by the fusion of monopotassium phosphates.[8] It is also prepared by dehydration of Potassium Phosphate.[6]

Potassium Phosphate

Food-grade potassium phosphates have been prepared by the neutralization of Phosphoric Acid with potassium hydroxide at 50 to 60°C.[9]

Potassium Polyphosphate

Potassium Polyphosphate can be obtained by heating monopotassium orthophosphate to any temperature above 150°C.[10]

Calcium Salts

Calcium Pyrophosphate

Calcium Pyrophosphate can be obtained by a solid state reaction (870˚C and normal atmosphere) from a mixture of Tricalcium Phosphate and Phosphoric Acid.[11] It can also be prepared by ignition of Dicalcium Phosphate.[6]

Dicalcium Phosphate

Commercial Dicalcium Phosphate is not a chemically-discrete entity, but is a mixture of varying amounts of dicalcium and monocalcium phosphates, Phosphoric Acid, calcium carbonate, and impurities, depending on the origin of the raw material and procedures employed in its industrial production.[12]

Tricalcium Phosphate

Tricalcium Phosphate has been produced by a calcination process (at high temperatures of 1500˚C to 1600˚C) that is preceded by the grinding and mixing of phosphate rock and sodium carbonate and the addition of Phosphoric Acid to the reaction mixture.[13]

Magnesium Salts

Magnesium Phosphate

Magnesium Phosphates have been prepared by adding a magnesium nitrate solution into mixed solutions of potassium hydroxide and Phosphoric Acid at temperatures of 29°C to 95°C.[14]

Composition/Impurities

Phosphoric Acid

According to the *Food Chemicals Codex* specification for this chemical, the following limits for inorganic impurities in Phosphoric Acid have been established: arsenic (≤ 3 mg/kg), cadmium (≤ 3 mg/kg), fluoride (≤ 10 mg/kg), and lead (≤ 3 mg/kg).[15]

Ammonium Salts

Ammonium Phosphate

According to the *Food Chemicals Codex* specification for Ammonium Phosphate, the acceptance criteria for this chemical indicate that it contains not less than 96% Ammonium Phosphate and not more than 102% Ammonium Phosphate. The following limits for inorganic impurities in Ammonium Phosphate have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 10 mg/kg), and lead (≤ 4 mg/kg).[15] According to another source, iron and aluminum have been mentioned as Ammonium Phosphate impurities.[16]

Diammonium Phosphate

According to the *Food Chemicals Codex* specification for Diammonium Phosphate, the acceptance criteria for this chemical indicate that it contains not less than 96% Diammonium Phosphate and not more than 102% Diammonium Phosphate. The following limits for inorganic impurities in Diammonium Phosphate have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 10 mg/kg), and lead (≤ 4 mg/kg).[15]

Sodium Salts

Sodium Hexametaphosphate

Sodium Hexametaphosphate contains 10 to 12 repeating pyrophosphate subunits.[17]

Sodium Phosphate

According to the *Food Chemicals Codex* specification for Sodium Phosphate, the acceptance criteria for this chemical is not less than 98% Sodium Phosphate and not more than 103% Sodium Phosphate on the dried basis, and the following limits for inorganic impurities have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 0.005%), and lead (≤ 4 mg/kg).[15]

Sodium Polyphosphate

According to the *Food Chemicals Codex* specification for Sodium Polyphosphate, the acceptance criteria for phosphorus pentoxide content range from 60% to 71%, and the following limits for inorganic impurities have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 0.005%), and lead (≤ 4 mg/kg).[15]

Trisodium Phosphate

According to the *Food Chemicals Codex* specification for Trisodium Phosphate, the acceptance criteria for this chemical are not less than 97% Trisodium Phosphate (anhydrous and monohydrate forms), calculated on the ignited basis, and not less than 90% Trisodium Phosphate (dodecahydrate), calculated on the ignited basis. The following limits for inorganic impurities have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 0.005%), and lead (≤ 4 mg/kg).[15]

Potassium Salts

Dipotassium Phosphate

According to the *Food Chemicals Codex* specification for Dipotassium Phosphate, the acceptance criteria for this chemical indicate that it contains no less than 98% Dipotassium Phosphate, on the dried basis. The following limits for inorganic impurities have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 10 mg/kg), and lead (≤ 2 mg/kg).[15] According to another source, heavy metal (as lead, 0.6×10^{-3} %) and arsenic (0.5×10^{-4} %) impurities have been reported for Dipotassium Phosphate.[18]

Potassium Metaphosphate

According to the *Food Chemicals Codex* specification for Potassium Metaphosphate, the acceptance criteria for this chemical are not less than 59% phosphorus pentoxide and no more than 61% phosphorus pentoxide. The following limits for inorganic impurities have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 10 mg/kg), and lead (≤ 2 mg/kg).[15]

Potassium Phosphate

According to the *Food Chemicals Codex* specification for Potassium Phosphate, the acceptance criteria for this chemical indicate that it contains not less than 98% Potassium Phosphate, on the dried basis. The following limits for inorganic impurities have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 10 mg/kg), and lead (≤ 2 mg/kg).[15]

Potassium Pyrophosphate

According to the *Food Chemicals Codex* specification for Potassium Pyrophosphate, the acceptance criteria for this chemical indicate that it contains no less than 95% Potassium Pyrophosphate. The following limits for inorganic impurities have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 10 mg/kg), and lead (≤ 2 mg/kg).[15]

Calcium Salts

Calcium Dihydrogen Phosphate

According to another source, Calcium Dihydrogen Phosphate may contain a trace amount of Phosphoric Acid as an impurity.[6]

Calcium Phosphate

Calcium Phosphate is approximately 96% pure, usually containing an excess of calcium oxide.[6]

Dicalcium Phosphate

Commercial Dicalcium Phosphate is not a chemically discrete entity, but is a mixture of varying amounts of dicalcium and monocalcium phosphates, Phosphoric Acid, calcium carbonate, and impurities, depending on the origin of the raw material and procedures employed in its industrial production.[12]

According to the *Food Chemicals Codex* specification for Dicalcium Phosphate, the acceptance criteria for this chemical indicate that it contains no less than 97% Dicalcium Phosphate and no more than 105% Dicalcium Phosphate (anhydrous or dehydrate form). The following limits for inorganic impurities have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 0.005%), and lead (≤ 2 mg/kg).[15]

Tricalcium Phosphate

According to the *Food Chemicals Codex* specification for Tricalcium Phosphate, the acceptance criteria for this chemical indicate that it contains not less than 34% Ca and not more than 40% Ca. The following limits for inorganic impurities have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 0.0075%), and lead (≤ 2 mg/kg).[15]

Magnesium Salts

Magnesium Hydrogen Phosphate

According to the *Food Chemicals Codex* specification for Magnesium Hydrogen Phosphate, the acceptance criteria for this chemical indicate that it contains not less than 96% $Mg_2P_2O_7$, on the ignited basis. The following limits for inorganic impurities have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 25 mg/kg), and lead (≤ 2 mg/kg).[15]

Trimagnesium Phosphate

According to the *Food Chemicals Codex* specification for Trimagnesium Phosphate, the acceptance criteria for this chemical indicate that it contains not less than 98% $Mg_3(PO_4)_2$ and not more than 101.5% $Mg_3(PO_4)_2$. The following limits for inorganic impurities have been established: arsenic (≤ 3 mg/kg), fluoride (≤ 25 mg/kg), and lead (≤ 2 mg/kg).[15]

USE

Cosmetic

The safety of Phosphoric Acid and its salts included in this assessment is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetic industry on the expected use of these ingredients 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. Use concentration data are submitted by Industry in response to surveys conducted by the Personal Care Products Council (Council) of maximum reported use concentrations, by product category. Collectively, the use frequency and use concentration data indicate that 22 of the 31 ingredients in this safety assessment are currently being used in cosmetic products (See Table 3). According to these data, the following 9 ingredients are not reported as being used in cosmetics:

Calcium Potassium Sodium Phosphate
Magnesium Hydrogen Phosphate
Magnesium Phosphate
Metaphosphoric Acid
Pentapotassium Triphosphate
Phosphate Buffered Saline
Potassium Polyphosphate
Sodium Polyphosphate
Sodium Trimetaphosphate

According to 2016 VCRP data, the greatest reported use frequency is for Phosphoric Acid (489 formulations, mostly rinse-off products), followed by Dicalcium Phosphate (327 formulations, mostly leave-on products) (Table 3).[19] The results of a concentration of use survey provided in 2015 indicate that Dicalcium Phosphate Dihydrate has the highest maximum concentration of use; it is used at concentrations up to 49% in rinse-off products (dentifrices) (Table 3).[20]

The highest maximum ingredient use concentration in leave-on products (10% in eye shadow) is being reported for Dicalcium Phosphate. In some cases, reported uses appear in the VCRP database, but concentrations of use data were not

provided; the opposite is also true. For example, according to the VCRP, Tetrapotassium Pyrophosphate and Calcium Pyrophosphate are being used in 95 and 3 cosmetic products, respectively; however, use concentration data on these ingredients were not provided in the concentration of use survey. Furthermore, use concentration data on Calcium Phosphate were provided in the concentration of use survey; however, use frequency data were not reported in the VCRP data.

Cosmetic products containing Phosphoric Acid or its salts may be applied to the skin and hair or, incidentally, may come in contact with the eyes (e.g., Dicalcium Phosphate at maximum use concentrations up to 10% in eye area cosmetics) and mucous membranes (e.g., Dicalcium Phosphate Dihydrate at maximum use concentrations up to 49% in dentifrices). Additionally, some of these ingredients are being used in products that may result in incidental ingestion. For example, Dicalcium Phosphate Dihydrate is being used in dentifrices at maximum use concentrations up to 49%, and Dicalcium Phosphate is being used in lipstick at maximum use concentrations up to 10%. Products containing these ingredients 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.

Phosphoric Acid is used in aerosol hair sprays at concentrations of < 0.01% and in pump hair sprays at concentrations up to 0.26%. The following other ingredients are also used in hair sprays: Potassium Phosphate (pump hair sprays up to 0.09%) and Sodium Phosphate (pump hair sprays up to 0.000014%). The following ingredients are used in face powders: Dicalcium Phosphate (up to 2.2%), Diammonium Phosphate (up to 0.00046%), Dicalcium Phosphate Dihydrate (up to 2.2%), Sodium Metaphosphate (up to 0.25%), and Sodium Phosphate (up to 0.086%). In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 μm, with propellant sprays yielding a greater fraction of droplets/particles below 10 μm, compared with pump sprays.[21,22,23,24] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[21,22] Additionally, Phosphoric Acid is used in dusting and talcum powders at concentrations up to 0.00001%, and Tricalcium Phosphate is used in dusting and talcum powders at concentrations up to 10%. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[25,26,27]

Noncosmetic

Phosphoric Acid and Phosphates

The U.S. FDA has determined that the following 20 ingredients included in this report are direct food additives that are generally recognized as safe (GRAS):[28]

Phosphoric Acid
Ammonium Phosphate
Calcium Dihydrogen Phosphate
Calcium Phosphate
Calcium Pyrophosphate
Diammonium Phosphate
Dicalcium Phosphate
Dipotassium Phosphate
Disodium Phosphate
Magnesium Hydrogen Phosphate
Pentasodium Triphosphate
Potassium Phosphate
Potassium Pyrophosphate
Sodium Hexametaphosphate
Sodium Metaphosphate
Sodium Phosphate
Sodium Trimetaphosphate
Tetrasodium Pyrophosphate
Trimagnesium Phosphate
Trisodium Phosphate

Additionally, the FDA has determined that potassium polymetaphosphate, chemically similar to one or more ingredients on the preceding list, is a GRAS direct food additive.

Acids

Phosphoric Acid

Phosphoric Acid is used in the manufacture of the following: phosphate salts, superphosphate fertilizers, detergents, activated carbon, animal feed, ceramics, dental cement, pharmaceuticals, soft drinks, gelatin, rust inhibitors, wax, and rubber latex.[3] Use in the following other processes has also been reported: electropolishing, engraving, photoengraving, lithograving, metal cleaning, sugar refining, and water treatment.

Metaphosphoric Acid

In dentistry, Metaphosphoric Acid is used to make zinc oxyphosphate cement.[6] It is also used as a reagent in chemical analysis.

Ammonium Salts

Ammonium Phosphate

In agriculture, Ammonium Phosphate has been an important granular fertilizer for many years.[29] Ammonium Phosphate is also used in dry chemical fire extinguishers, which are commonly found in offices, schools, and homes. The extinguisher spray disperses finely powdered Ammonium Phosphate, which coats the fuel and rapidly smothers the flame.

Diammonium Phosphate

Diammonium Phosphate is a complex fertilizer that contains 2 major nutrients, nitrogen and phosphorus.[30] Additionally, Diammonium Phosphate is used in fireproofing textiles, paper, wood, vegetable fibers, and dentifrices.[6]

Sodium Salts

Disodium Phosphate

Disodium Phosphate is used as an emulsifier and buffer in foods, and in the manufacture of enamels, ceramics, detergents, and boiler compounds.[6]

Disodium Pyrophosphate

Disodium Pyrophosphate is used chiefly in baking powders.[6]

Pentasodium Triphosphate

Pentasodium Triphosphate is used as a preservative, sequestrant, and texturizer in foods, and as whitening agent in toothpaste; it is also used in water softeners and detergents.[6]

Sodium Hexametaphosphate

Sodium Hexametaphosphate is an anti-tartar ingredient in toothpaste, and is known to remove stains.[17]

Sodium Phosphate

Sodium phosphate products have been used for bowel cleansing prior to medical procedures such as colonoscopy. The FDA is aware of reports of acute phosphate nephropathy that are associated with such usages.[31] Acute phosphate nephropathy is a form of acute kidney injury that is associated with deposits of calcium phosphate crystals in the renal tubules, which may result in permanent renal function impairment. In response, FDA requires that the manufacturer of 2 oral sodium phosphate products (prescription only) for bowel cleansing add a Boxed Warning to the labeling for these products. The FDA has also stated that, in light of the risk of acute phosphate nephropathy, over-the-counter laxative oral sodium phosphate products should not be used for bowel cleansing.

Sodium Phosphate is also used in baking powders and as dry acidulant and sequestrant for foods.[6]

Sodium Polyphosphate, Sodium Trimetaphosphate, and Tetrasodium Pyrophosphate

Blended phosphates (usually ortho and glassy polyphosphates) are used in municipal water treatment as part of scale-control and corrosion-control programs in the United States, because these compounds bind calcium carbonate, iron, magnesium, and manganese.[32] Sodium Polyphosphate**,** Sodium Trimetaphosphate, and Tetrasodium Pyrophosphate are some of the chemicals that are found in the phosphate blends. Sodium Trimetaphosphate is also used in detergent processing, and as a crosslinking agent for starch in foods and pharmaceuticals.[6]

Tetrasodium Pyrophosphate is also used in processed meat products, as an emulsifier in cheese, and as a color preservative in soybean paste.[33] Other uses include: sequestrant, dispersant, deflocculant, detergent builder, and component of solid or liquid fertilizers.[34] Tetrasodium Pyrophosphate is one of the anti-calculus components of most tartar control dentifrices that are marketed.[35]

The United States Environmental Protection Agency (EPA) has established an exemption from the requirement of a tolerance for residues of Tetrasodium Pyrophosphate when used as an inert ingredient in pesticide formulations applied to growing crops only.[36]

Trisodium Phosphate

Trisodium Phosphate is used in photographic developers, in detergent mixtures, and in the manufacture of paper.[6]

Potassium Salts

Dipotassium Phosphate

Dipotassium Phosphate is used as a buffering agent in antifreeze, nutrient in the culturing of antibiotics, ingredient of instant fertilizers, and as a sequestrant in the preparation of non-dairy powdered coffee creams.[6]

Potassium Phosphate

Potassium Phosphate is used as a buffering agent in pharmaceuticals.[6]

Tetrapotassium Pyrophosphate

Blended phosphates (usually ortho and glassy polyphosphates) are used in municipal water treatment as part of scale-control and corrosion-control programs in the United States, because these compounds bind calcium carbonate, iron, magnesium, and manganese.[32] Sodium Polyphosphate**,** Sodium Trimetaphosphate, and Tetrasodium Pyrophosphate are some of the chemicals that are found in the phosphate blends. Sodium Trimetaphosphate is also used in detergent processing, and as a crosslinking agent for starch in foods and pharmaceuticals.[6]

Calcium Salts

Calcium Phosphate

Calcium Phosphate has been used as an adjuvant (i.e., a material that can increase the humoral or cellular immune response to an antigen) for simultaneous immunizations with diphtheria, tetanus, polio, Bacillus Calmette-Guerin (BCG), yellow fever, measles and hepatitis B vaccines, with hepatitis B and DTP-polio vaccines, and immunization with allergens.[37] It has also been used in the manufacture of fertilizers, Phosphoric Acid, P compounds, milk-glass, polishing and dental powders, porcelains, and pottery.[6]

Calcium Phosphate is an active ingredient in antacid over-the-counter (OTC) drug products that are generally recognized as safe and effective.[38]

Calcium Pyrophosphate

One form of Calcium Pyrophosphate has been used clinically as a bone-graft extender, because it bonds with host bone.[39] It is also used in dentifrices and in the production of ceramic ware and glass.[6]

Dicalcium Phosphate

Dicalcium Phosphate is used chiefly in animal feeds, and is also used as a mineral supplement in cereals and other foods.[6]

Dicalcium Phosphate Dihydrate

Dicalcium Phosphate Dihydrate is a cleaning and polishing agent that is specifically used in dentifrices that contain monofluorophosphate.[40] As an abrasive, this ingredient assists in the removal of dental stains and deposits that form on tooth surfaces.

The FDA has determined that there are inadequate data to establish general recognition of the safety and effectiveness of Dicalcium Phosphate Dihydrate as an active ingredient in anticaries OTC drug products**.**[38]

Tricalcium Phosphate

Tricalcium Phosphate, described as a porous ceramic material, is used in bone transplantation surgery.[41] It acts as a scaffold for bone ingrowth, undergoing progressive degradation and replacement by bone. Most often, it is used in granule or powder form during surgery.

Tricalcium Phosphate is an active ingredient in antacid OTC drug products, and FDA has established a maximum daily dosage limit of 24 grams for Tricalcium Phosphate in these products.[42]

Magnesium Salts

Magnesium Hydrogen Phosphate and Trimagnesium Phosphate

The FDA has determined that Magnesium Hydrogen Phosphate and Trimagnesium Phosphate are GRAS as a direct human food ingredients.[43]

TOXICOKINETICS

Phosphorus (as phosphate) is an essential constituent of all known protoplasms, and its content is uniform across most plant and animal tissues.[44] According to the 1994 United States Department of Agriculture (USDA) survey of food intake of individuals, values for the mean daily phosphorus intake from food were 1,495 mg (males, ≥ 9 years) and 1,024 mg (females, ≥ 9 years). In both sexes, intakes decreased at age ≥ 51 years.

Structurally, phosphorus occurs as phospholipids, which constitute a major component of most biological membranes, and as components as nucleotides and nucleic acids. The total phosphorus concentration in whole blood is 13 mmol/liter (40 mg/dl), most of which is in the phospholipids of red blood cells and plasma lipoproteins. Approximately 1 mmol/liter (3.1 mg/dl) is present as inorganic phosphate (P_i), which is a tiny fraction of body phosphorus (< 0.1 %). In adults, P_i makes up approximately 15 mmol (465 mg) of body phosphorus, and is located mainly in the blood and

extracellular fluid. Phosphate enters the P_i pool during absorption from the diet and resorption from bone, and is the primary source from which cells of all tissues derive both structural and high-energy phosphate.[44] Furthermore, most of the urinary phosphorus and hydroxyapatite mineral phosphorus are derived from the P_i.

Phosphates are absorbed from the gastrointestinal tract, and the transport of phosphate from the lumen is an active, energy-dependent process; vitamin D stimulates phosphate absorption.[45] At physiologic pH (7.4), extracellular phosphate is present primarily as the Disodium Phosphate and Sodium Phosphate (4:1). Once absorbed, phosphate combines with calcium to form Dicalcium Phosphate in bones and teeth.[32] Free orthophosphate is the primary form by which dietary P_i is absorbed. When phosphate ion is ingested in very large amounts, most of the phosphate ion uptake from the gut is eliminated in the feces.[46] According to another source, approximately two thirds of the ingested phosphate is absorbed from the gastrointestinal tract in adults, and absorbed phosphate is almost entirely excreted in the urine.[45]

ANIMAL

Phosphoric Acid

Phosphoric Acid dissociates and is then absorbed as phosphate and hydronium ions through mucous membranes.[47]

Sodium Salts

Sodium Hexametaphosphate

Sodium Hexametaphosphate is converted to Sodium Phosphate in the stomach.[48]

After hexametaphosphate was administered to rats and rabbits by stomach tube, no more than trace amounts of labile phosphate were found in the urine.[8,49]

Sodium Polyphosphate

Ingested polyphosphates are degraded by phosphatase enzymes to monophosphates.[32] The short- and long-chain polyphosphates are absorbed intact only to a very limited extent, if at all, and the larger molecules are hydrolyzed by phosphatases (present in the gut) to monophosphates.[50]

In an animal study (number and species not stated), 10% to 30% of administered Sodium Polyphosphate was absorbed as monophosphate, and small amounts of oligophosphates were found in the urine.[8] In another experiment in which labeled Sodium Polyphosphate was administered to rats, the chemical was not absorbed as such, but was taken up, after hydrolysis, as monophosphate and diphosphate. In 18 h, 40% of the dose was hydrolyzed and absorbed.[8,51]

Potassium Salts

Potassium Metaphosphate

In an animal study (species and number not stated), 10% to 30% of administered Potassium Metaphosphate was absorbed as monophosphate, and small amounts of oligophosphates were found in the urine.[52] Study details were not provided.

When radiolabeled (radiolabel not specified) Potassium Metaphosphate was administered orally to rats, approximately half of the radioactivity was recovered from the feces, mainly as polymeric phosphate. Only a small percentage of the dose was found in the urine, in the form of monophosphate.[52]

HUMAN

Sodium Salts

Sodium Phosphate

In a pharmacokinetic analysis, 45 ml of a laxative containing 30 g of Sodium Phosphate was administered to 13 normal volunteers.[53,54,55] The subjects were divided into the following 2 groups: Group 1 (median weight = 60 kg) and Group 2 (median weight = 119.2 kg). Serum and urine electrolytes were measured for 12 h. Hydration was maintained by monitoring the weight, fluid intake, and total body water. Markedly elevated serum phosphate levels were observed in Group 1, compared to Group 2. The normalized area under the phosphate vs. time curve was much higher in Group 1 (1120 ± 190 mg/dl · min) than in Group 2 (685 ± 136 mg/dl · min); $P < 0.001$was reported for this comparison. The urinary excretion of calcium was significantly lower in Group 1 (mean $=16.4 \pm 7.6$ mg), compared to Group 2 (mean $= 39.2 \pm 7.8$ mg); $P < 0.001$ was reported for this comparison. The results of this study demonstrated that lower body-weight individuals develop prolonged high serum phosphate levels after ingesting Sodium Phosphate. The authors noted that individuals of lower body weight are at risk for acute phosphate nephropathy when they use colonoscopy preparations containing Sodium Phosphate.

Calcium Salts

Tricalcium Phosphate

The absorption of ingested Tricalcium Phosphate was evaluated in 10 women. The subjects ingested Tricalcium Phosphate (1200 mg) after fasting for 12 h.[56,57] Calcium and phosphorus absorption were determined by the postload rise in urinary calcium and phosphate, respectively, above baseline. A statistically significant increase in urinary calcium excretion ($P < 0.001$) was observed during the 2-4 h post-load period, and a statistically significant increase in serum calcium ($P < 0.02$) was observed at 4 h post-load. Statistically significant increases in urinary phosphate excretion ($P < 0.001$) and serum phosphorus ($P < 0.001$) were also reported.

TOXICOLOGY

Calcium Phosphate

The English abstract of a Japanese publication on the safety of a Calcium Phosphate bone paste was available.[58] The following series of tests was performed: acute toxicity, pyrogenicity, hemolysis, intracutaneous reactivity, sensitization, genotoxicity, and cytotoxicity. The authors noted that there was no evidence of abnormal or toxic effects in any of these tests. The abstract does not include pertinent details relating to study results.

Single Dose (Acute) Toxicity

ANIMAL

DERMAL

Phosphoric Acid and Salts

Results of acute dermal studies for Phosphoric Acid and its salts are presented in Table 6. In studies involving rabbits, an LD_{50} of 2740 mg/kg and an LD_{50}> 3160 mg/kg were reported for Phosphoric Acid. For ammonium salts of phosphoric acid, the reported LD_{50} was > 5000 mg/kg (rats) and ranged from > 7940 mg/kg to > 10,000 mg/kg (rabbits). LD_{50} values ranging from > 300 mg/kg to > 7940 mg/kg (rabbits) were reported for sodium salts of phosphoric acid. The dermal administration of potassium salts of phosphoric acid to rabbits resulted in reported LD_{50} values ranging from > 300 mg/kg to > 10,000 mg/kg. LD_{50} values ranging from > 300 mg/kg to > 7940 mg/kg were reported for calcium salts of phosphoric acid. Reported LD_{50} values ranging from > 2000 mg/kg to > 7940 mg/kg were reported for magnesium salts of phosphoric acid.

ORAL

Phosphoric Acid and Salts

Acute oral LD_{50} values for Phosphoric Acid and its salts are presented in Table 5. In studies involving rats, the LD_{50} for Phosphoric Acid ranged from 1530 mg/kg to 4400 mg/kg. The LD_{50} for Phosphoric Acid in rabbits was 2740 mg/kg. The oral LD_{50} for the ammonium salts of phosphoric acid in studies involving rats ranged from 3250 mg/kg (Ammonium Phosphate) to > 25,100 mg/kg (Diammonium Phosphate). Sodium salts of phosphoric acid were administered to rats, mice, hamsters and guinea pigs in acute oral toxicity studies, and the LD_{50} ranged from 1300 mg/kg (Tetrasodium Pyrophosphate [mice]) to 10,600 mg/kg (Sodium Trimetaphosphate [rats]). For potassium salts of phosphoric acid administered orally in studies involving rats or mice, the acute oral LD_{50} ranged from 1,000 mg/kg (Tetrapotassium Pyrophosphate [mice]) to 7,100 mg/kg (Potassium Phosphate [rats]). In acute oral toxicity studies on calcium salts of phosphoric acid involving rats or mice, the reported LD_{50} ranged from 2,170 mg/kg (Calcium Phosphate [rats]) to > 10,000 mg/kg (Calcium Pyrophosphate [rats]). The reported LD_{50} for Magnesium Phosphate in studies involving rats ranged from > 1,000 mg/kg (Magnesium Phosphate) to > 10,000 mg/kg (Trimagnesium Phosphate).

INHALATION

Phosphoric Acid and Salts

Acute inhalation toxicity data on Phosphoric Acid and its sodium, potassium, and calcium salts are presented in Table 4. At the highest lethal concentrations tested, Phosphoric Acid caused tracheal lesions in rabbits, rats, and mice, but not in guinea pigs. Due to its hygroscopic nature, Phosphoric Acid aerosols will combine with water molecules in the air within the human tracheobronchial tree, which increases the aerodynamic diameter of the particles of the aerosol. This effect is known as hygroscopic growth. As a result, the deposition characteristics of these aerosols change as they pass through the respiratory tract, which will affect the total deliverable dose in the lungs after inhalation.[47] Overall, the data suggest that the sodium, potassium, and calcium salts of Phosphoric Acid exhibit a low potential for inhalation toxicity.

According to one publication, Phosphoric Acid caused moderate to acute inhalation toxicity in mice.[59] Pertinent details were not included in this BIBRA Toxicity Profile abstract on phosphoric acid and common inorganic phosphates.

Short-Term, Subchronic, and Chronic Toxicity Studies

ORAL

The results of short-term, subchronic, and chronic oral toxicity studies on Phosphoric Acid and its salts are summarized in Table 7. In the longest duration feeding study on Phosphoric Acid, a no-observed-effect level (NOEL) of 338 mg/kg/day was reported for rats that received concentrations up to 0.75% in the diet for one year. The average weight of the parathyroid glands (only parameter assessed) was 235% of control values in rabbits that received oral doses of Diammonium Phosphate up to 700 mg/kg/day for up to 16 months. Kidney damage (nephrocalcinosis) was a common pathological finding in repeated oral dose toxicity studies involving sodium and potassium salts of Phosphoric Acid. There were basically no adverse effects in rats/monkeys fed calcium salts of Phosphoric Acid in the diet.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY

Reproductive and developmental toxicity data on ammonium, sodium, potassium, and calcium salts of Phosphoric Acid are presented in Table 8. Teratogenicity was assessed primarily using rats and mice; however, rabbits and hamsters were also used. These salts did not produce teratogenic effects *in vivo*, and the highest dose tested was 1500 mg/kg/day Diammonium Phosphate (in rats) for 35 days. However, the following salts of Phosphoric Acid were teratogenic to chick embryos: Tetrasodium Pyrophosphate (injection of 5 mg/egg), Sodium Hexametaphosphate (injection of 0.5 to 10 mg/egg), Sodium Phosphate (injection of 0.5 to 10 mg/egg), Potassium Phosphate (injection of 10 mg/egg), Calcium Phosphate (injection of 2.5 mg/egg), and Tricalcium Phosphate (injection of 2.5 mg/egg). Information relating to whether or not pH was measured or controlled in the eggs was not found.

GENOTOXICITY

The in vitro and in vivo genotoxicity data on Phosphoric Acid and its ammonium, sodium, potassium, and calcium salts are presented in Table 9. The in vitro tests included the Ames/*Salmonella* mutagenicity assay (with and without metabolic activation), the *Saccharomyces cerevisiae* mutagenicity assay (with and without metabolic activation), the chromosome aberrations assay (Chinese hamster fibroblasts), and the in vitro cytogenetics assay (human lung cells). The in vivo tests included the dominant lethal test (rats), host-mediated assay (mice), and the mouse translocation test. Phosphoric Acid and its ammonium, sodium, potassium, and calcium salts did not produce positive responses in in vitro or in vivo genotoxicity assays.

CARCINOGENICITY

ANIMAL

Acids

Phosphoric Acid

According to one source, no carcinogenic potential was demonstrated in limited feeding studies involving rats treated with Phosphoric Acid or several of its salts. However, in rodents treated orally, several phosphates have been shown to promote the effects of known carcinogenicity.[59] Pertinent details were not included in this BIBRA Toxicity Profile abstract on phosphoric acid and common inorganic phosphates.

Sodium Salts

Disodium Phosphate and Tetrasodium Pyrophosphate

An oral feeding study involving groups of 10 male and 10 female rats fed various concentrations of a mixed preparation (33% Potassium Metaphosphate + 67% Tetrasodium Pyrophosphate [in Sherman diet]) was conducted.[8,60] The following diets were fed to the rats:

- 0.5% commercial preparation (effective concentration [Potassium Metaphosphate] = 0.5% x 33% = 0.17%; effective concentration [Tetrasodium Pyrophosphate] = 0.5% x 67% = 0.34%)
- 1% commercial preparation (effective concentration [Potassium Metaphosphate] = 1% x 33% = 0.33%; effective concentration [Tetrasodium Pyrophosphate] = 1% x 67% = 0.67%)
- 5% commercial preparation (effective concentration [Potassium Metaphosphate] = 5% x 33% = 1.7%; effective concentration [Tetrasodium Pyrophosphate] = 5% x 67% = 3.4%)

From each dietary group, a second and third generation were produced and feeding was continued. For all dietary groups, the tumor incidence was not greater than that observed in control animals. Additional study details were not provided.

Pentasodium Triphosphate

Groups of weanling rats of the Rochester strain (number not stated) were maintained on a diet supplemented with 0.05%, 0.5%, or 5% Pentasodium Triphosphate for 2 years.[61] The carcinogenesis indexes were similar to the frequencies expected for aging rats, and did not vary among dietary groups.

Sodium Hexametaphosphate

Groups of weanling rats (males and females; number and strain not stated) were fed a diet containing 0.05%, 0.5%, or 5% Sodium Hexametaphosphate for 2 years.[61] There was no correlation between the dietary level of Sodium Hexametaphosphate and tumor incidence.

Sodium Trimetaphosphate

A diet containing 0.1%, 1%, or 10% Sodium Trimetaphosphate was fed to groups of weanling rats (number and strain not stated) for 2 years. There was no correlation between the dietary level of Sodium Trimetaphosphate and tumor incidence.[61]

Sodium Metaphosphate

Calcium sodium metaphosphate (CSM) fiber is a manmade inorganic fiber composed of condensed polyphosphate chains in a specific crystal lattice.[62] Male and female Fischer 344 rats (80/sex/group) were exposed (inhalation) to CSM fiber 6 h/day, 5 days/week at target-exposure levels of 0, 1, 5, or 25 mg/m^3 (corresponding to 0, 27, 80, and 513 fibers/cc, respectively) for 24 months. At 3 and 12 months, 10 rats/sex/group were killed and, at 18 and 24 months, 5 rats/sex/group were killed. Additionally, 5 rats/sex/group were removed from exposure at 18 months and maintained for a 6-month recovery period. No increase in tumors (benign or malignant) was observed in this study.

Tumor Promotion

Potassium Salts

Dipotassium Phosphate

In a tumor promotion study involving groups of 20 uni-nephrectomized male rats, the following diets were used:[18,63]

- Group I: 1,000 ppm *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) diet (2 weeks) + 50,000 ppm Dipotassium Phosphate diet (18 weeks)
- Group II: Basal diet (2 weeks) + 50,000 ppm Dipotassium Phosphate(18 weeks)
- Group III: 1,000 ppm EHEN diet (2 weeks) + the basal diet (18 weeks)
- Group IV: Basal diet (20 weeks)

The rats were fed EHEN (1,000 ppm) in the diet for 2 weeks, and the left kidney was removed at week 3. After nephrectomy, the rats were fed Dipotassium Phosphate (50,000 ppm) in the diet for 18 weeks (from weeks 3 to week 20). A control group of 20 rats received basal diet only after EHEN administration and nephrectomy. The mean relative kidney weight per body weight in group I was significantly greater when compared to group III. Additionally, the mean kidney weight in group II was significantly greater when compared to group IV. The numbers of simple hyperplastic foci and adenomatous hyperplastic foci in group I animals were statistically significantly greater ($p < 0.05$) when compared to group III. The incidence of renal cell tumors was 30% in group I. Nephropathy, lymphocyte accumulation, hyaline droplets in proximal convoluted tubular cells, and dilatation of the proximal convoluted tubular cells were observed in the cortex of group I and group II animals. Calcification was observed in the renal medulla and cortex of groups I and II. It was concluded that Dipotassium Phosphate promoted the development of renal tubular cell tumors. The authors noted that the results documented in this study clearly suggest a link between toxicity-dependent proliferation and promoting ability.

In a medium-term bioassay for renal tumorigenesis, the feeding of male Wistar rats with 5% potassium dibasic phosphate in the diet promoted the development of preneoplastic lesions.[64] These study results were obtained from the limited details found in the English abstract of a Japanese publication.

Phosphate

A study was performed to elucidate the potential effects of high dietary phosphate (P_i) on the development of lung cancer.[65] The first experiment involved two groups of male K-*ras*^{LA1} mice (9 per group). One group received an AIN93-based diet containing 0.5% P_i (normal P_i), and the other group received the same diet fortified with 1% P_i (high P_i). Both diets were fed to the mice for 4 weeks, after which the animals were killed. Blood samples were obtained and necropsy was performed. Tumor lesions of lung surfaces were counted and the diameter of each lesion was measured. A lobe of the left lung was prepared for histopathological examination and immunohistochemistry. The diet containing 1% P_i increased lung tumor progression and growth, when compared with the diet containing 0.5% P_i. Histopathological examination results showed that pulmonary tumor progression was markedly stimulated by 1% P_i in the diet. The number and size (at least 1.5 mm in diameter) of lung surface tumor lesions (adenomas) increased significantly ($P < 0.05$). P_i (1%) in the diet also had the

following effects: (1) increased the sodium-dependent inorganic phosphate transporter-2b protein levels in the lungs; (2) stimulated pulmonary protein kinase B (Akt; known to regulate cell cycle progression) activity, while suppressing (a) the protein levels of tumor suppressor phosphatase and tensin homolog deleted on chromosome 10 and (b) the Akt binding partner carboxyl-terminal modulator protein, resulting in facilitated cap-dependent protein translation; and (3) significantly ($P < 0.05$) stimulated cell proliferation in the lungs of K-*ras*^{LA1}mice.

In a second study (urethane-induced lung cancer model), A/J mice were injected intraperitoneally with urethane (1 mg/g body weight) in saline. At 4 weeks post-injection, the mice were divided into 2 groups (7 mice per group) and fed 1% P_i and 0.5% P_i in the diet, respectively, for 4 weeks. The effect of high dietary P_i on lung tumorigenesis was confirmed in this experiment. P_i (1%) in the diet statistically significantly increased ($P < 0.05$) tumor development. Both the mean number of tumors and the mean tumor diameter (at least 1 mm in diameter) increased statistically significantly ($P < 0.05$). Histopathological examination results also showed that pulmonary tumor progression was stimulated. The authors noted that the results of this study indicate that high dietary P_i strongly activated Akt signaling and increased lung tumorigenesis.[65]

OTHER RELEVANT STUDIES

Cytotoxicity

Calcium Phosphate and Dicalcium Phosphate Dihydrate

The cytotoxicity of the following mixture was evaluated using a mouse L-929 cell suspension: Tricalcium Phosphate (90%; α-) and Dicalcium Phosphate Dihydrate (10%) in a solution containing chondroitin sulfate (5%) and sodium succinate (12%).[66] Cell morphology was evaluated at 24 h; the affected area of the cell layer was determined using microscopy. Contracted cells, rounded cells with dark nuclei, and broken cells were considered damaged cells. A very low degree of cytotoxicity (mild cytotoxicity) was observed.

Calcium Pyrophosphate

The cytotoxicity of Calcium Pyrophosphate was studied using Chinese hamster ovary K-1 cells.[11] Cytotoxicity potential was determined quantitatively by cytolethality (expressed as the cytotoxicity index [$IC_{50\%}$]) using a colony suppression assay. The $IC_{50\%}$ is defined as the concentration that is necessary to kill half of the cell population or the concentration that suppresses colony formation to 50% of the control value. Phenol solution (0.02%) and alumina extracts served as positive and negative controls, respectively. Calcium Pyrophosphate was not cytotoxic ($IC_{50\%} = 100$). The positive and negative controls performed as expected.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Skin irritation and sensitization data on Phosphoric Acid and its ammonium, sodium, potassium, calcium, and magnesium salts are presented in Table 10. A broad range of reactions (from irritation/no irritation (Phosphoric acid and salts) to irritating/corrosive (Phosphoric Acid) effects) reported for phosphoric acid or its salts at concentrations within and outside of the range of those used in cosmetic products. Phosphoric acid was an irritant at concentrations as low as 2.5%; however, the pH of the test substance was low, pH of 2.1.[67] The corrosive effect of Phosphoric Acid was observed at concentrations ranging from 17.5% (pH of 0.6 to 0.2) to 100%.[50,67] The sodium salts were non-irritating (Sodium Phosphate)[50] to moderately irritating (Disodium Phosphate)[50], and the potassium and calcium salts were non-irritating (Potassium Phosphate and Dicalcium Phosphate)[50] to mildly irritating (Dipotassium Phosphate and Calcium Phosphate)[50] to rabbit skin. The ammonium salts (Ammonium Phosphate and Diammonium Phosphate) were non-irritating to mildly irritating to rabbit skin.[50] The magnesium salts of Phosphoric Acid (Magnesium Phosphate and Trimagnesium Phosphate) were non-irritating to the skin of rabbits.[50] Pentasodium Triphosphate (50% solution) and Sodium Metaphosphate (1% solution) were mildly irritating to the skin of human subjects.[32] Phosphoric Acid was not sensitizing in human subjects,[50,68] and Sodium Phosphate (10% in propylene glycol) was not sensitizing in the local lymph node assay.[69]

OCULAR IRRITATION

ANIMAL

Ocular irritation data on phosphoric acid and its ammonium, sodium, potassium, calcium, and magnesium salts are presented in Table 11. Phosphoric Acid was corrosive to the eyes of rabbits at concentrations in the 70% - 85% range,[50, 70,71] but was non-irritating at concentrations of 10% and 17%.[67,70] None of the salts of Phosphoric Acid was found to be corrosive to the eyes of rabbits. However, ocular irritation was observed; for example, Tetrasodium Pyrophosphate was irritating at a concentration of 10% and Trisodium Phosphate was irritating at concentrations of 10% and 15%.[32,50]

MUCOSAL IRRITATION

HUMAN

Phosphoric Acid

Phosphoric Acid (50%) was applied to the gingival tissue and teeth of 26 orthodontic patients.[3] The 90-second contact period for the acid was followed by rinsing. No demonstrable test substance-related effect on treated tissues was observed during the 7-day observation period.

Tetrasodium Pyrophosphate

Tetrapotassium Pyrophosphate

Some non-prescription dentifrices, particularly pyrophosphate-based tartar control toothpastes, may be irritating to oral tissues.[35] The following clinical observations were made in patients (number not stated) at a dental clinic that frequently uses tartar control toothpastes containing Tetrasodium Pyrophosphate and/or Tetrapotassium Pyrophosphate: pale gingival tissues, mucosal sloughing, small blisters, dryness of oral tissues, and/or free-gingival-margin erythema. Subjective symptoms included a painful, burning sensation of oral tissues (most commonly gingival mucosa); a generalized, non-specific sensitivity or odd feeling to teeth and/or soft tissues; and sensations of "itchy" oral tissues. Patient complaints averaged approximately 5 per week over a 2-year period. Amelioration of the patients' chief symptoms occurred rapidly upon switching to a non-tartar control toothpaste.

CLINICAL REPORTS

Calcium Pyrophosphate

The articular deposition of Calcium Pyrophosphate (Calcium Pyrophosphate deposition disease [CPPD]) is a common age-related phenomenon. Frequently, this disease is asymptomatic and unassociated with structural joint damage.[72,73] Acute attacks of synovitis, resulting in pseudogout, are observed.[74] These attacks are often provoked by local trauma or surgery and commonly involve the knee, and, less often, the wrist, hip, shoulder, and elbow.

Sodium Phosphate

A systematic review of adverse event reports relating to oral Sodium Phosphate (used for bowel cleansing prior to colonoscopy) was performed.[75] Fifty-eight publications of significant events in 109 patients who used Sodium Phosphate were identified. Between January of 2006 and December of 2007, the most commonly reported findings were electrolyte disturbances, renal failure, and colonic ulceration. The number of cases of renal failure reported to FDA during this period was 171.

A retrospective study of renal adverse event reports was performed using the FDA Adverse Event Reporting System, a voluntary reporting system available for public access.[76] A total of 2,097,223 files (years 2004–2008 and the first 9 months of 2009) from FDA's website were examined. Of the 178 patients (71% women) on sodium phosphate tablets identified, an increasing number of renal adverse drug reactions associated with tablet preparations were reported each year. In 2006, nine of 74 (12%) renal adverse drug reactions (ADRs) were reported to be from ingesting tablets ; results for subsequent years were as follows: 40 of 181 (22%) [2007], 46 of 148 (31%) [2008], and 60 of 795 (7.55%) [2009]. The mean weight for women with renal complications from tablet preparations was 68.57 ± 1.78 kg, statistically significantly lower than the national average weight of 74 ± 0.5 kg for the same age group ($P = 0.003$) in the National Health and Nutrition Examination Survey. It was concluded that renal adverse drug reactions from sodium phosphate tablets were more common in women with a mean body weight lower than the national average weight.

In more recent studies, 10 adult cases of acute phosphate nephropathy, associated with acute renal failure, following administration of a Sodium Phosphate preparation before colonoscopy, and a case series of 3 children with severe hyperphosphatemia and hypocalcemia after the use of Sodium Phosphate-containing laxatives were reported.[77,78] Acute renal failure due to phosphate nephropathy following bowel cleansing with an oral Sodium Phosphate solution was reported in another patient.[79] Electron microscopy of a kidney biopsy sample revealed membranous glomerulonephritis and Calcium Phosphate deposits were observed in tubular cells and in tubules. Phosphate remained elevated for 11 days; other electrolyte levels were normal. A biopsy taken only 2 months before the acute kidney disease showed no sign of the Calcium Phosphate deposits in the second biopsy. It was concluded that the phosphate load given to the patient was responsible for the biopsy findings.

EPIDEMIOLOGY

Acids

Phosphoric Acid

In the 1980s, a large population-based case-control study in Montreal was performed to explore the possible associations among hundreds of occupational substances and multiple cancer sites,[80] and an analysis of the occupational information collected in this study (focusing on renal cell cancer) was subsequently performed.[80] In this study, the following individuals were interviewed: 142 male patients with pathologically confirmed renal carcinoma; 1900 controls with cancer at other sites; and 533 population-based controls. Logistic regression results for exposure to selected substances were presented, including the following 2 sets of odds ratios: (1) OR_1 (95% confidence interval [CI]): Odds ratios (adjusted for respondent status, age, smoking and body mass index [BMI]) and 95% CI; (2) OR_2 (95%CI): Odds ratios (adjusted for respondent status, age, smoking, BMI and occupational confounders) and 95% CI. The authors concluded that there was evidence of excess risk for renal cell carcinoma following workplace exposure to Phosphoric Acid, as indicated by the following odds ratios: The OR_1 value reported for phosphoric acid was 3.4 (1.3-9.2), and an OR_2 value of 2.4 (0.8-7.0) was reported.[80]

In the International Agency for Research on Cancer (IARC) monograph on occupational exposures to mists and vapors from sulfuric acid and other inorganic acids (including Phosphoric Acid), several questionable epidemiological studies in the phosphate fertilizer manufacturing industry showed excess lung cancer; but, IARC did not classify Phosphoric Acid as carcinogenic.[81] However, IARC did conclude that occupational exposure to strong-inorganic-acid mists containing sulfuric acid is carcinogenic to humans.

Phosphates

Cancer morbidity and mortality were studied in a population of employees of phosphate ore mining and processing operations in Central Florida.[82] The workers involved in the study were employed by participating phosphates companies between 1950 and 1979, and the study population consisted of 3541 male employees who had worked for 6 months or more. Based upon an industrial hygiene analysis, only drying/shipping, chemical/fertilizer, and maintenance job categories were found to have the potential for exposure to high levels of dust, chemical fumes, or radiation. Cancer incidence was traced using questionnaires confirmed by medical records, and by tumor registry searches. Standardized incidence ratios (SIRs) were calculated. To estimate the study population's risk in relation to general rates in the United States, standardized mortality ratios (SMRs) adjusted for age and calendar time were calculated. The SMRs were tested for statistical significance at the 0.05 level using the Poisson distribution. Statistically significant elevations in lung cancer (standardized mortality ratio = 1.62) and emphysema were observed when compared to rates in the United States. For workers employed over a period of 20 years, there was a dose-response trend of increasing lung cancer risk with increasing duration of employment (standardized mortality ratio = 2.48, with 20 years of employment). There was no evidence of excess lung cancer risk among employees who were hired after 1960. The authors noted that multivariate analyses and internal comparisons of risk by job type were consistent with a hypothesis of occupationally-related lung cancer, but that the small numbers prevented firm conclusions.

RISK ASSESSMENT

Phosphates, Diphosphates, and Polyphosphates

ORAL

Phosphates, diphosphates (i.e., pyrophosphates), and polyphosphates (e.g., metaphosphates) were evaluated by the Joint FAO/WHO Expert Committee on Food Additives. [83] A maximum tolerable daily intake (MTDI) of 70 mg/kg was determined, based on the lowest concentration of phosphorus (6600 mg/day) that caused nephrocalcinosis in rats. "The MTDI is expressed as phosphorus and applies to the sum of phosphates naturally present in food and the phosphates derived from use of these food additives." The FAO/WHO Expert Committee considered establishing an average daily intake (ADI) to be inappropriate because phosphorus (as phosphates) is an essential nutrient and an unavoidable constituent of food. The Federation of American Societies for Experimental Biology (FASEB) estimate of maximum tolerable daily intake of phosphates in man is also 70 mg/kg.[84]

INHALATION

Phosphoric Acid

The EPA calculated an inhalation reference concentration (RfC) of 1×10^{-2} mg/m^3 for Phosphoric Acid (the critical effect is bronchiolar fibrosis).[85] Development of an inhalation RfC involves evaluating toxic effects inside the respiratory system (port-of-entry effects) and outside the respiratory system (extra-respiratory effects). In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects over a lifetime of exposure. The

calculated RfC for Phosphoric Acid is based on inhalation toxicity data summarized in the Repeated Dose Toxicity Section of this safety assessment.[86] Based on the histologic lesions in the tracheobronchiolar region, 180 mg/m^3 was the LOAEL, and 50 mg/m^3 was the NOAEL in this study.

SUMMARY

The safety of 31 ingredients, Phosphoric Acid and its salts, as used in cosmetics is reviewed in this safety assessment. The functions of these ingredients in cosmetic products frequently include buffering agents, corrosion inhibitors, chelating agents, and pH adjusters.

According to the 2016 VCRP data, the greatest reported use frequency is for Phosphoric Acid (489 formulations, mostly rinse-off), followed by Dicalcium Phosphate (327 formulations, mostly leave-on). Lower use frequencies were reported for the remaining salts. The results of a concentration of use survey provided in 2015 indicate that Dicalcium Phosphate Dihydrate has the highest maximum concentration of use; it is used at concentrations up to 49% in rinse-off products (dentifrices).

Phosphoric Acid ionizes and is then absorbed as phosphate and hydronium ions through mucous membranes. Some of the phosphate and hydronium ions are conjugated in the liver and then excreted in the urine. Following the absorption of phosphates from the gastrointestinal tract, phosphate combines with calcium to form calcium hydrogen orthophosphate in bones and teeth. Free orthophosphate is the primary form by which dietary P_i is absorbed. In general, approximately two thirds of the ingested phosphate is absorbed from the gastrointestinal tract in adults, and absorbed phosphate is almost entirely excreted in the urine.

In acute inhalation toxicity studies, at the highest lethal concentrations, Phosphoric Acid caused tracheal lesions in rabbits, rats, and mice, but not in guinea pigs. Overall, the data suggest that the sodium, potassium, and calcium salts exhibit a low potential for inhalation toxicity. The EPA has calculated an inhalation reference concentration (RfC) of 1 x 10^{-2} mg/m^3 for Phosphoric Acid, based on the results from two parallel 13-week inhalation toxicity studies involving rats. In general, the RfC is an estimate of a daily inhalation exposure of the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime.

In acute oral toxicity studies involving rats, the LD_{50} for Phosphoric Acid ranged from 1530 mg/kg to 4400 mg/kg. The oral LD_{50} for Phosphoric Acid in rabbits was 2740 mg/kg. The oral LD_{50} for the ammonium salts of Phosphoric Acid in studies involving rats ranged from 5750 mg/kg (Ammonium Phosphate) to > 25,100 mg/kg (Diammonium Phosphate). Sodium salts of Phosphoric Acid were administered to rats, mice, hamsters and guinea pigs in acute oral toxicity studies, and LD_{50} values ranged from 1300 mg/kg (Tetrasodium Pyrophosphate (mice)) to 10,600 mg/kg (Sodium Trimetaphosphate [rats]). For potassium salts of Phosphoric Acid administered orally in studies involving rats or mice, acute oral LD_{50} values ranged from 1,000 mg/kg (Tetrapotassium Pyrophosphate, involving mice) to 7,100 mg/kg (Potassium Phosphate [rats]). In acute oral toxicity studies on calcium salts of Phosphoric Acid involving rats or mice, LD_{50} values ranged from 2,170 mg/kg (Calcium Phosphate [rats]) to > 10,000 mg/kg (Calcium Pyrophosphate (rats)). LD_{50} values for Magnesium Phosphate in studies involving rats ranged from > 1,000 mg/kg (Magnesium Phosphate) to > 10,000 mg/kg (Trimagnesium Phosphate).

The feeding of Phosphoric Acid at concentrations up to 0.75% in the diet of rats for 52 weeks yielded a NOEL of 338 mg/kg/day. A NOAEL of 105 mg/kg/day was reported in a study in which sheep received doses of Phosphoric Acid up to 211 mg/kg/day for 70 days. A NOAEL of 250 mg/kg/day was reported for groups of rats that received Diammonium Phosphate at doses up to 1500 mg/kg/day for 35 days. The average weight of the parathyroid glands (only parameter assessed) was 235% of control values in rabbits that received oral doses of Diammonium Phosphate up to 700 mg/kg/day for up to 16 months.

A study of rats fed Disodium Phosphate or Disodium Pyrophosphate (up to 5% in the diet) for 100 days resulted in an LOEL (renal histopathology) of < 2571 mg/kg/day (Disodium Phosphate) and an LOEL (renal histopathology) = 450 mg/kg/day (Disodium Pyrophosphate). When Disodium Phosphate, Pentasodium Triphosphate, or Tetrasodium Pyrophosphate was administered to rats at concentrations up to 5% in the diet for 39 weeks, a LOEL of 495 mg/kg/day was reported. Of the NOELs determined in rat studies, the highest NOEL (338 mg/kg/day) was reported in a study in which rats were fed Phosphoric Acid at concentrations up to 0.75% in the diet daily for> 52 weeks. The highest NOAEL (2623 mg/kg/day) was reported in a study in which rats were fed Dipotassium Phosphate at concentrations up to 5.1% in the diet daily for 150 days. In studies involving dogs, a NOAEL of 100 mg/kg/day was reported for the following sodium salts, each of which was administered orally at a dose of 100 mg/kg/day for 30 days: Pentasodium Triphosphate, Sodium Polyphosphate/Sodium Hexametaphosphate, and Sodium Trimetaphosphate. Kidney damage (nephrocalcinosis) was a common finding in repeated dose oral toxicity studies involving sodium salts of Phosphoric Acid. The feeding of commercial preparations, to rats, containing effective concentrations of up to 3.4% Tetrasodium Pyrophosphate and 1.7% Potassium Metaphosphate also resulted in nephrocalcinosis.

When potassium salts of Phosphoric Acid were fed in the diet of rats at concentrations ranging from 0.6% to 10%, nephrocalcinosis/nephrotoxicity was observed at concentrations of 5% (Tetrapotassium Pyrophosphate (daily doses; number

of days not stated)) and 10% (Tetrapotassium Pyrophosphate [daily doses; number of days not stated] or Dipotassium Phosphate (8 weeks)). Nephrocalcinosis was also observed in dogs that were fed a diet providing Dipotassium Phosphate at a dose of 800 mg/kg/day. There were basically no adverse findings in rats/monkeys fed calcium salts of Phosphoric Acid in the diet (up to 0.8% calcium and 1.30% phosphorus). The same was true for rats that received Dicalcium Phosphate or Tricalcium Phosphate at doses up to 1000 mg/kg/day.

In acute dermal toxicity studies involving rabbits, an LD_{50} of 2740 mg/kg and an LD_{50} > 3160 mg/kg were reported for Phosphoric Acid. For ammonium salts of Phosphoric Acid, the dermal LD_{50} for rats was > 5000 mg/kg (rats) and ranged from > 7940 mg/kg to > 10,000 mg/kg for rabbits. Dermal LD_{50} values ranging from > 300 mg/kg to > 7940 mg/kg (rabbits) were reported for sodium salts of Phosphoric Acid. The dermal administration of potassium salts of Phosphoric Acid to rabbits resulted in dermal LD_{50} values ranging from > 300 mg/kg to > 10,000 mg/kg. Dermal LD_{50} values ranging from > 300 mg/kg to > 7940 mg/kg were reported for calcium salts of Phosphoric Acid. LD_{50} values ranging from > 2000 mg/kg to > 7940 mg/kg were reported for magnesium salts of Phosphoric Acid.

The teratogenicity of ammonium, sodium, potassium, and calcium salts of Phosphoric Acid was assessed primarily using rats and mice; however, rabbits and hamsters were also used. These salts did not produce teratogenic effects *in vivo*, and the highest dose tested was Diammonium Phosphate at 1500 mg/kg/day for 35 days. However, the following salts of Phosphoric Acid were teratogenic to chick embryos: Tetrasodium Pyrophosphate (injection of 5 mg/egg), Sodium Hexametaphosphate (injection of 0.5 to 10 mg/egg), Sodium Phosphate (injection of 0.5 to 10 mg/egg), Potassium Phosphate (injection of 10 mg/egg), Calcium Phosphate (injection of 2.5 mg/egg), and Tricalcium Phosphate (injection of 2.5 mg/egg).

Both *in vitro* and *in vivo* genotoxicity data on Phosphoric Acid and its ammonium, sodium, potassium, and calcium salts are available. The *in vitro* tests included the Ames/*Salmonella* mutagenicity assay (with and without metabolic activation), the *Saccharomyces cerevisiae* mutagenicity assay (with and without metabolic activation), the chromosome aberrations assay (Chinese hamster fibroblasts), and the *in vitro* cytogenetics assay (human lung cells). The *in vivo* tests included the dominant lethal test (rats), host-mediated assay (mice), and the mouse translocation test. Phosphoric Acid and its ammonium, sodium, potassium, and calcium salts did not produce positive responses in *in vitro* or *in vivo* genotoxicity assays.

In an oral carcinogenicity study, rats were fed mixtures containing up to 1.7% Potassium Metaphosphate and up to 5% Tetrasodium Pyrophosphate. Feeding was continued through the second and third generations produced. For all dietary groups, the tumor incidence was similar to control animals. When groups of rats were fed Pentasodium Triphosphate or Sodium Hexametaphosphate at concentrations up to 5% for 2 years, there was no correlation between concentration in the diet and tumor incidence. The same was true for rats fed a diet containing up to 10% Sodium Trimetaphosphate.

The results of a study on high dietary P_i intake and the development of lung cancer in mice indicated that high dietary P_i strongly activated Akt signaling and increased lung tumorigenesis

In a population-based case-control study, workplace exposure to Phosphoric Acid produced some evidence of excess risk of renal cell carcinoma. Furthermore, in an IARC monograph on occupational exposure to Phosphoric Acid and other inorganic acids, there were several questionable epidemiological studies of the phosphate fertilizer manufacturing industry that showed excess lung cancer. However, IARC did not classify Phosphoric Acid as carcinogenic. Dipotassium Phosphate, given in the diet (containing the carcinogen, EHEN) of male rats, promoted the development of renal tumors.

Skin irritation and sensitization data on Phosphoric Acid and its ammonium, sodium, potassium, calcium, and magnesium salts are available, and a broad range of reactions (non-irritating to corrosive) have been reported. Phosphoric Acid was classified as non-irritating or corrosive. Phosphoric acid was an irritant at concentrations as low as 2.5%; however, the pH of the test substance was low, pH of 2.1. The corrosive effect of Phosphoric Acid was observed at concentrations ranging from 17.5% (pH of 0.6 to 0.2) to 100%, but 19% Phosphoric Acid was non-irritating. The sodium salts were non-irritating to moderately irritating, and the potassium and calcium salts were non-irritating to mildly irritating to rabbit skin. The magnesium salts of Phosphoric Acid were non-irritating to the skin of rabbits. Pentasodium Triphosphate and Sodium Metaphosphate were mildly irritating to the skin of human subjects. Phosphoric Acid was a non-sensitizer in human subjects, and Sodium Phosphate was a non-sensitizer in the local lymph node assay.

Phosphoric Acid was corrosive to the eyes of rabbits at concentrations in the 70% - 85% range, but was non-irritating at concentrations of 10% and 17%. None of the salts of Phosphoric Acid was found to be corrosive to the eyes of rabbits. However, ocular irritation was observed; for example, Tetrasodium Pyrophosphate was irritating at a concentration of 10% and Trisodium Phosphate was irritating at concentrations of 10% and 15%.

Renal failure has resulted from the use of sodium-phosphate-containing colonoscopy preparations. Other case reports have indicated that some non-prescription dentifrices, particularly pyrophosphate-based tartar control toothpastes, may be irritating (erythema, burning, and mucosal sloughing) to oral tissues. The clinical findings relate to tartar control toothpastes containing Tetrasodium Pyrophosphate and/or Tetrapotassium Pyrophosphate.

DISCUSSION

The Panel noted the broad range of results (from irritation/no irritation to irritating/corrosive effects) reported for Phosphoric Acid or its salts at concentrations within and outside of the range of those used in cosmetic products. The results of a concentration of use survey provided by the Council in 2015 indicate that Dicalcium Phosphate Dihydrate has the highest maximum concentration of use; it is used at concentrations up to 49% in rinse-off products (dentifrices). Phosphoric acid was an irritant at concentrations as low as 2.5%; however, the pH of the test substance was low, pH of 2.1. The corrosive effect of Phosphoric Acid was observed at concentrations ranging from 17.5% (pH of 0.6 to 0.2) to 100%. For salts of Phosphoric Acid, skin irritation was observed at concentrations ranging from 1% to 50% and ocular irritation was observed at concentrations as low as 10% and 15%.

The Panel noted that test animals fed high concentrations of Phosphoric Acid in the diet exhibited renal damage and evidence of the tumor-promoting potential of Phosphoric Acid. The oral exposures to Potassium Phosphate in one of these studies promoted the development of kidney tumors initiated by treatment with a potent renal carcinogen. The Panel also discussed animal studies on Potassium Phosphate indicating that this salt was not associated with renal damage or cancer, and one epidemiological study suggesting an association between occupational exposures to Phosphoric Acid and kidney and lung cancer. The Panel concluded that renal toxicity and tumor promotion would not be expected from exposures to cosmetic products containing phosphoric acid or its salts, because such exposures can reasonably be anticipated to be substantially lower than those associated with adverse effects in these studies.

Concern about heavy metals that may be present in salts of Phosphoric Acid was expressed by the Panel. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities in the ingredient before blending into cosmetic formulations.

The Panel discussed the issue of incidental inhalation exposure from propellant and pump hair sprays and face powders. The Panel considered inhalation toxicity data and pertinent data indicating that incidental inhalation exposures to these ingredients in such cosmetic products would not cause adverse health effects, including acute inhalation toxicity data on Phosphoric Acid and its salts and data characterizing the potential for these ingredients to cause acute and repeated dose oral toxicity, and ocular or dermal irritation or sensitization. The Panel noted that droplets/particles from spray and loose-powder cosmetic products would not be respirable to any appreciable amount. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

CONCLUSION

The CIR Expert Panel concluded that the following 31 ingredients are safe in the present practices of use and concentration in cosmetics as described in this safety assessment, when formulated to be non-irritating.

Phosphoric Acid
Ammonium Phosphate
Dicalcium Phosphate
Calcium Dihydrogen Phosphate
Calcium Phosphate
Calcium Potassium Sodium Phosphate*
Calcium Pyrophosphate
Diammonium Phosphate
Dicalcium Phosphate Dihydrate
Dipotassium Phosphate
Disodium Phosphate
Disodium Pyrophosphate
Magnesium Hydrogen Phosphate*
Magnesium Phosphate*
Metaphosphoric Acid*
Pentapotassium Triphosphate
Pentasodium Triphosphate*
Phosphate Buffered Saline*
Potassium Metaphosphate
Potassium Phosphate
Potassium Polyphosphate*
Sodium Hexametaphosphate
Sodium Metaphosphate
Sodium Polyphosphate*
Sodium Phosphate
Sodium Trimetaphosphate*
Tetrapotassium Pyrophosphate
Tetrasodium Pyrophosphate
Tricalcium Phosphate
Trimagnesium Phosphate
Trisodium Phosphate

*Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.

TABLES

Table 1. Definitions, Structures, and functions of the ingredients in this safety assessment.[1,6]

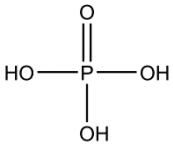
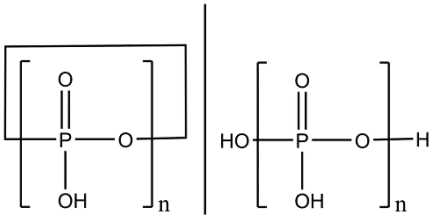
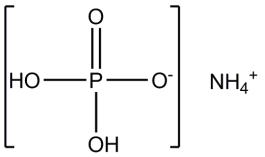
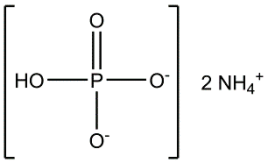
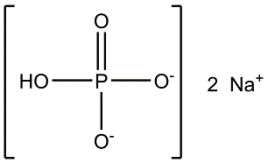
Ingredient/CAS No.	Definition & Structure	Function
Acids		
Phosphoric Acid 7664-38-2	Phosphoric Acid is the inorganic acid that conforms to the formula:  [Commonly called orthophosphoric acid]	Fragrance Ingredients; pH Adjusters
Metaphosphoric Acid 10343-62-1 37267-86-0	Metaphosphoric Acid is the inorganic acid that conforms to the formula:  ["Metaphosphoric" is a term used for a series of condensed protonated phosphates prepared by dehydration of orthophosphates; differing reaction conditions lead to various cyclic or linear polymeric structures. True metaphosphates, with the general formula, $(MHPO_3)n$, are cyclic polymers. Commonly "n" is 3.]	pH Adjusters
Ammonium Salts		
Ammonium Phosphate 7722-76-1	Ammonium Phosphate is an inorganic salt that conforms to the formula:  [Commonly called ammonium dihydrogen orthophosphate]	Buffering Agents; Oral Care Agents; pH Adjusters
Diammonium Phosphate 7783-28-0	Diammonium Phosphate is the inorganic salt that conforms to the formula:  [Commonly called ammonium hydrogen orthophosphate]	Buffering Agents; Corrosion Inhibitors; Oral Care Agents
Sodium Salts		
Disodium Phosphate 10140-65-5 7558-79-4 7782-85-6	Disodium Phosphate is the inorganic salt that conforms to the formula:  [Commonly called disodium hydrogen orthophosphate]	Buffering Agents; Corrosion Inhibitors; Fragrance Ingredients; pH Adjusters

Table 1. Definitions, Structures, and functions of the ingredients in this safety assessment.[1,6]

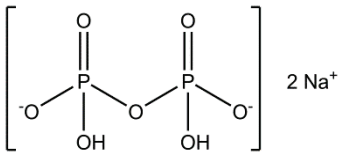
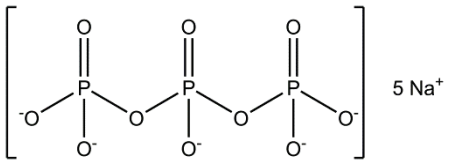
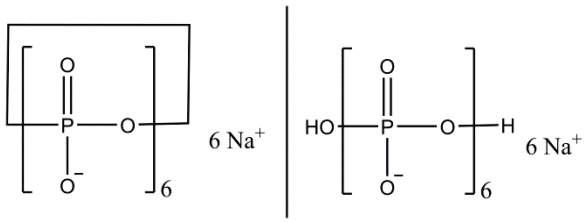
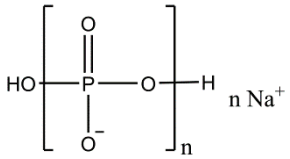
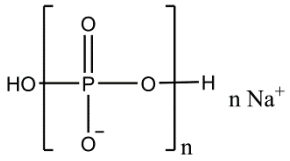
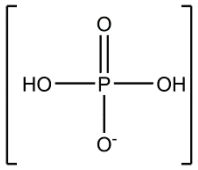
Ingredient/CAS No.	Definition & Structure	Function
Disodium Pyrophosphate 7758-16-9	Disodium Pyrophosphate is the inorganic salt that conforms generally to the formula:  [Commonly called disodium dihydrogen pyrophosphate]	Buffering Agents; Chelating Agents; Corrosion Inhibitors; pH Adjusters
Pentasodium Triphosphate 7758-29-4	Pentasodium Triphosphate is the inorganic salt that conforms to the formula:  [Commonly called pentasodium metaphosphate]	Chelating Agents; pH Adjusters
Sodium Hexametaphosphate 10124-56-8 10361-03-2 68915-31-1	Sodium Hexametaphosphate is the inorganic salt that conforms generally to the formula:  [The name, Sodium Hexametaphosphate, has been used for both the cyclic hexamer and for a mixture of soluble Sodium Phosphate polymers also known as sodium polymetaphosphate.]	Chelating Agents; Corrosion Inhibitors; Fragrance Ingredients
Sodium Metaphosphate 10361-03-2 50813-16-6	Sodium Metaphosphate is a linear Sodium Polyphosphate that conforms generally to the formula:  ["Metaphosphate" is a term used for a series of condensed inorganic phosphates prepared by dehydration of orthophosphates; differing reaction conditions lead to various cyclic or linear polymeric structures. In contrast with the definition of this ingredient, true metaphosphates, with the general formula, $(MPO_3)n$, are cyclic polymers. Commonly "n" is 3.]	Chelating Agents; Oral Care Agents
Sodium Polyphosphate 68915-31-1	Sodium Polyphosphate is a mixture of the sodium salts of Polyphosphoric Acid. 	Chelating Agents
Sodium Phosphate 7558-80-7 7632-05-5	Sodium Phosphate is the inorganic salt that conforms to the formula:  [Commonly referred to as sodium orthophosphate]	Buffering Agents

Table 1. Definitions, Structures, and functions of the ingredients in this safety assessment.[1,6]

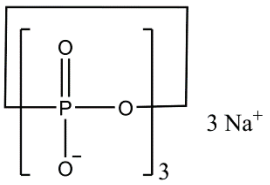
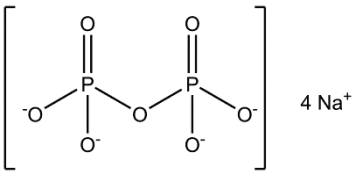
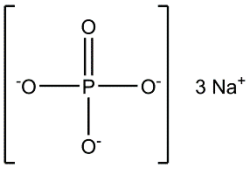
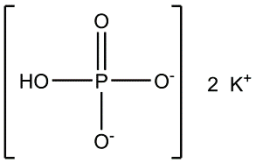
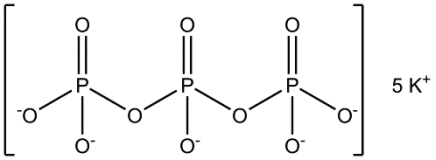
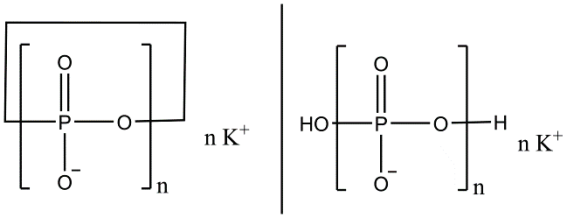
Ingredient/CAS No.	Definition & Structure	Function
Sodium Trimetaphosphate 7785-84-4	Sodium Trimetaphosphate is the inorganic salt that conforms to the formula:  [“Metaphosphate” is a term used for a series of condensed inorganic phosphates prepared by dehydration of orthophosphates; differing reaction conditions lead to various cyclic or linear polymeric structures. True metaphosphates, with the general formula, $(MPO_3)n$, are cyclic polymers and “n” is 3.]	Buffering Agents; Chelating Agents; pH Adjusters
Tetrasodium Pyrophosphate 7722-88-5	Tetrasodium Pyrophosphate is the inorganic salt that conforms to the formula: 	Buffering Agents; Chelating Agents; Corrosion Inhibitors; Oral Care Agents; pH Adjusters
Trisodium Phosphate 7601-54-9	Trisodium Phosphate is the inorganic salt that conforms to the formula:  [Commonly referred to as trisodium orthophosphate]	Chelating Agents; pH Adjusters
Potassium Salts		
Dipotassium Phosphate 7758-11-4	Dipotassium Phosphate is the inorganic salt that conforms generally to the formula:  [Commonly called dipotassium hydrogen orthophosphate]	Corrosion Inhibitors; pH Adjusters
Pentapotassium Triphosphate 13845-36-8	Pentapotassium Triphosphate is the inorganic salt that conforms to the formula:  [Commonly called pentapotassium metaphosphate]	Chelating Agents; pH Adjusters
Potassium Metaphosphate 7790-53-6	Potassium Metaphosphate is the potassium salt of Metaphosphoric Acid.  [“Metaphosphate” is a term used for a series of condensed inorganic phosphates prepared by dehydration of orthophosphates; differing reaction conditions lead to various cyclic or linear polymeric structures. True metaphosphates, with the general formula, $(MPO_3)n$, are cyclic polymers. Commonly “n” is 3.]	Surfactants - Cleansing Agents

Table 1. Definitions, Structures, and functions of the ingredients in this safety assessment.[1,6]

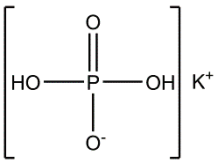
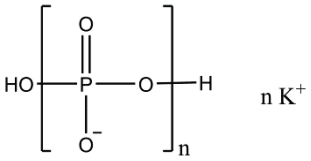
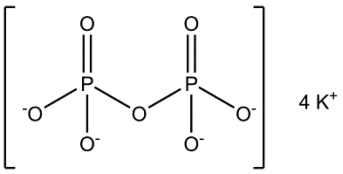
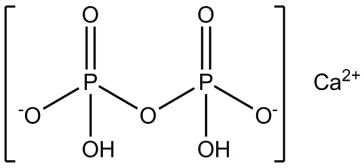
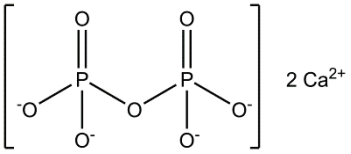
Ingredient/CAS No.	Definition & Structure	Function
Potassium Phosphate 16068-46-5 7778-77-0	Potassium Phosphate is the inorganic salt that conforms generally to the formula:  [commonly called potassium dihydrogen orthophosphate]	pH Adjusters
Potassium Polyphosphate 68956-75-2	Potassium Polyphosphate is the potassium salt of Polyphosphoric Acid. 	Chelating Agents
Tetrapotassium Pyrophosphate 7320-34-5	Tetrapotassium Pyrophosphate is the inorganic salt that conforms to the formula: 	Buffering Agents; Chelating Agents; Corrosion Inhibitors; Oral Care Agents; pH Adjusters
Calcium Salts		
Calcium Dihydrogen Phosphate 7758-23-8	Calcium Dihydrogen Phosphate is the inorganic salt that conforms to the formula: [Commonly called calcium dihydrogen orthophosphate]	pH Adjusters
Calcium Phosphate 10103-46-5	Calcium Phosphate is the inorganic salt that conforms to the formula:  [Though a representative structure is drawn (commonly called calcium dihydrogen pyrophosphate), the actual ratio of phosphate (with various degrees of protonation) to calcium is unknown, as is the form of phosphate, for this ingredient]	Abrasives; Buffering Agents; Bulking Agents; Oral Care Agents
Calcium Pyrophosphate 7790-76-3	Calcium Pyrophosphate is the inorganic salt that conforms to the formula:  [Commonly called dicalcium pyrophosphate]	Abrasives; Buffering Agents; Bulking Agents; Oral Care Agents

Table 1. Definitions, Structures, and functions of the ingredients in this safety assessment.[1,6]

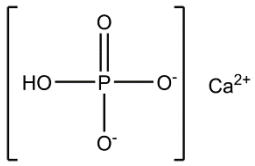
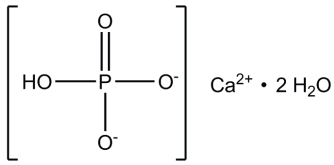
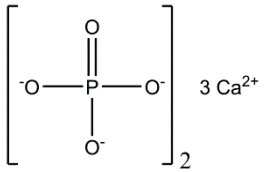
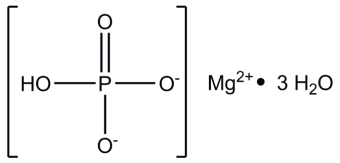
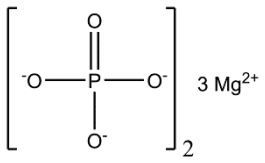
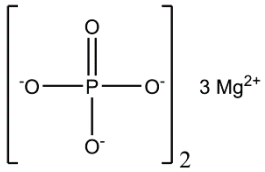
Ingredient/CAS No.	Definition & Structure	Function
Dicalcium Phosphate 7757-93-9	Dicalcium Phosphate is the inorganic salt that conforms to the formula:  [Commonly called calcium hydrogen orthophosphate]	Abrasives; Opacifying Agents; Oral Care Agents
Dicalcium Phosphate Dihydrate 7789-77-7	Dicalcium PhosphateDihydrate is the inorganic salt that conforms to the formula:  [Commonly called calcium hydrogen orthophosphate dihydrate]	Abrasives; Opacifying Agents; Oral Care Agents
Tricalcium Phosphate 7758-87-4	Tricalcium Phosphate is the inorganic salt that consists of a variable mixture of Calcium Phosphates having the approximate composition:  [Commonly called tricalcium orthophosphate]	Abrasives; Fragrance Ingredients; Opacifying Agents; Oral Care Agents
Magnesium Salts		
Magnesium Hydrogen Phosphate 7782-75-4	Magnesium Hydrogen Phosphate is the inorganic salt that conforms to the formula:  [Commonly called magnesium hydrogen orthophosphate trihydrate]	Anticaking Agents
Magnesium Phosphate 10043-83-1	Magnesium Phosphate is the inorganic salt that conforms to the formula:  Though a representative structure is drawn (commonly called trimagnesium orthophosphate), the actual ratio of phosphate (with various degrees of protonation) to magnesium is unknown, as is the form of phosphate (i.e., ortho, pyro, or meta), for this ingredient]	Dispersing Agents - Nonsurfactant
Trimagnesium Phosphate 7757-87-1	Trimagnesium Phosphate is the inorganic salt that conforms to the formula:  [Commonly called trimagnesium orthophosphate]	Bulking Agents; Opacifying Agents
Multi-cation Salts		

Table 1. Definitions, Structures, and functions of the ingredients in this safety assessment.[1,6]

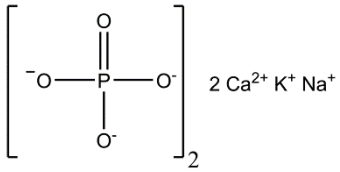
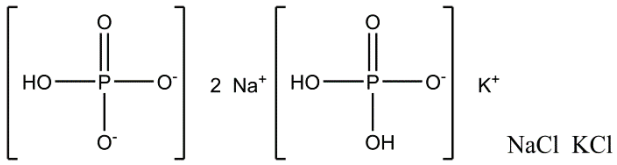
Ingredient/CAS No.	Definition & Structure	Function
Calcium Potassium Sodium Phosphate 131862-42-5	Calcium Potassium Sodium Phosphate is the inorganic salt produced by the reaction of sodium carbonate, potassium carbonate and calcium hydrogen phosphate.  [Commonly called dicalcium potassium sodium orthophosphate]	Abrasives; Anticaries Agents; Antimicrobial Agents; Oral Care Agents
Phosphate Buffered Saline	Phosphate Buffered Saline is a phosphate buffered solution containing a physiological concentration of inorganic salt. [It is an aqueous solution containing phosphate and chloride salts of sodium, potassium, calcium, or magnesium (or some combination thereof). For example, Phosphate Buffered Saline (PBS) solutions, by one protocol, may contain: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4. However, no submission has been received that indicates which protocol(s) utilized in the cosmetic ingredient, Phosphate Buffered Saline.][87]  	Solvents

Table 2. Properties Phosphoric Acid and its Salts.[6]

Property	Value	Background Information
	Phosphoric Acid	
Form	Unstable orthorhombic crystals or clear, syrupy liquid	
% Composition	H (3.09%), O (65.31%), and P (31.61%)	
Formula weight	97.99	
Density	1.8741 (100% solution)	
Solubility	Miscible with water and alcohol. Soluble in 8 vols of a 3:1 ether:alcohol mixture	
Melting point	42.35°C (orthorhombic crystals)	Becomes anhydrous at 150°. Changes to Metaphosphoric Acid when heated above 300°.
	Metaphosphoric Acid	
Form	Transparent, glass-like solid or soft silky masses; hygroscopic	Volatilizes at red heat
% Composition	H (1.26%), O (60.01%), and P (38.7%)	
Formula weight	79.98	
Solubility	Very slowly soluble in cold water, slowly changing to H_3PO_4. Soluble in alcohol	
	Ammonium Phosphate	
Form	Odorless crystals or white crystalline powder	Stable in air
% Composition	H (5.26%), N (12.18%), O (55.64%), and P (26.93%)	
Density	1.80	
Formula weight	115.02	
Solubility	1 g dissolves in ~ 2.5 ml water; slightly soluble in alcohol; practically insoluble in acetone	
Boiling point	376.1°C	
Melting point	193.3°C	
	Calcium Dihydrogen Phosphate	
Form	Monohydrate, large, shining, triclinic plates, crystalline powder, or granules	Non-hygroscopic when pure, but traces of impurities such as H_3PO_4 cause material to be deliquescent. Loses H_2O at 100°. Decomposes at 200°
% Composition	Ca (17.12%), H (1.72%), O (54.69%), and P (26.47%)	
Density	2.220	
Formula weight	234.05	

Table 2. Properties Phosphoric Acid and its Salts.[6]

Property	Value	Background Information
	Calcium Dihydrogen Phosphate	
Solubility	Moderately soluble in water; soluble in dilute HCl or HNO_3 or acetic acid	
	Calcium Pyrophosphate	
Form	Polymorphous crystals or powder	
% Composition	Ca (31.55%), O (44.07%), and P (24.38%)	
Density	3.09	
Formula weight	254.10	
Solubility	Practically insoluble in water; soluble in dilute HCl or HNO_3	
	Diammonium Phosphate	
Form	Odorless crystals or crystalline powder	Gradually loses approximately 8% NH_3 upon exposure to air
% Composition	H (6.87%), N (21.21%), O (48.46%), and P (23.45%)	
Formula weight	132.06	
Solubility	1 g dissolves in 1.7 ml water; practically insoluble in alcohol and acetone	
	Dialcium Phosphate	
Form	Triclinic crystals	At red heat, dehydrated to Calcium Pyrophosphate
% Composition	Ca (29.46%), H (0.74%), O (47.04%), and P (22.76%)	
Formula weight	136.06	
Solubility	Soluble in 3N HCl or 2N HNO_3; practically insoluble in water and alcohol	
	Dicalcium Phosphate Dihydrate	
Form	Monoclinic crystals	Loses water of crystallization slowly below 100°. Dehydration at red heat to Calcium Pyrophosphate
Density	2.31	
Solubility	Slightly soluble in dilute acetic acid; soluble in dilute HCl or HNO_3; practically insoluble in water and alcohol	

Table 2. Properties Phosphoric Acid and its Salts.[6]

Property	Value	Background Information
	Dipotassium Phosphate	
Form	White, hydroscopic granules	Converted into pyrophosphate by ignition
% Composition	H (0.58%), K (44.90%), O (36.74%), and P (17.78%)	
Formula weight	174.17	
	Dipotassium Phosphate	
Solubility	Very soluble in water; slightly soluble in alcohol	
	Disodium Phosphate	
Form	Hygroscopic powder	On exposure to air, will absorb from 2 to 7 mols H_2O, depending on the humidity and temperature
% Composition	H (0.71%), Na (32.39%), O (45.08%), and P (21.82%)	
Formula weight	141.96	
Solubility	Soluble in water; insoluble in alcohol	
	Disodium Pyrophosphate	
Form	White fused masses or powders	Decomposes at 220°
% Composition	H (0.91%), Na (20.72%), O (50.46%), and P (27.91%)	
Solubility	Soluble in water	
	Magnesium Hydrogen Phosphate	
Form	White crystalline powder	
% Composition	H (0.84%), Mg (20.21%), O (53.21%), and P (25.75%)	
Density	2.13	
Formula weight	120.28	
Solubility	Soluble in dilute acids; slightly soluble in water	

Table 2. Properties Phosphoric Acid and its Salts.[6]

Property	Value	Background Information
	Magnesium Phosphate	
Form	White powder	
% Composition	H (1.85%), Mg (11.13%), O (58.64%), and P (28.38%)	
Formula weight	218.28	
Solubility	Soluble in water	
	Pentasodium Triphosphate	
Form	Slightly hygroscopic granules	Reverts to the orthophosphate with prolonged heating
% Composition	Na (31.25%), O (43.49%), and P (25.26%)	
Formula weight	367.86	
Solubility	Soluble in water	
	Potassium Metaphosphate	
Form	White, monoclinic crystals	
Density	2.45	
Solubility	Soluble in aqueous solutions of alkali metal (except potassium) salts; insoluble in water	
	Potassium Phosphate	
Form	Colorless crystals or white, granular powder	At 400˚, loses H_2O, forming metaphosphate
% Composition	H (1.48%), K (28.73%), O (47.03%), and P (22.76%)	
Density	2.34	
Formula weight	136.08	
Solubility	Soluble in water; practically insoluble in alcohol	

Table 2. Properties Phosphoric Acid and its Salts.[6]

Property	Value	Background Information
	Potassium Polyphosphate	
Form	White, monoclinic crystals	
Density	2.45	
Solubility	Soluble in aqueous solutions of alkali metals (except potassium) salts; insoluble in water	
	Sodium Hexametaphosphate	
		The name, Sodium Hexametaphosphate, has been used for both the cyclic hexamer and for a mixture of soluble Sodium Phosphate polymers
	Sodium Metaphosphate	
		The name, Sodium Metaphosphate, is used for a series of condensed inorganic phosphates prepared by the dehydration of sodium orthophosphates
	Sodium Phosphate	
% Composition	H (1.68%), Na (19.16%), O (53.34%), and P (25.82%)	
Formula weight	119.98	
	Sodium Polyphosphate	
Form	Clear, hygroscopic glass	Depolymerizes in aqueous solution to form Sodium Trimetaphosphate and sodium orthophosphates
Solubility	Soluble in water	
Melting point	628˚C	
	Sodium Trimetaphosphate	
Form	White crystals or white, crystalline powder	Hydrolyzes to sodium tripolyphosphate (Pentasodium Triphosphate) in dilute alkaline solution
% Composition	Na (22.55%) O (47.07%), and P (30.38%)	
Density	2.49	
Solubility	Soluble in water	

Table 2. Properties Phosphoric Acid and its Salts.[6]

Property	Value	Background Information
	Tetrapotassium Pyrophosphate	
% Composition	K (47.34%), O (33.90%), and P (18.75%)	
Formula weight	330.33	
Solubility	Soluble in water; insoluble in alcohol	
	Tetrasodium Pyrophosphate	
Form	Crystals	Hydrolyzes to orthophosphate in aqueous solution
% Composition	Na (34.58%), O (42.12%), and P (23.30%)	
Density	2.534	
Formula weight	265.90	
Solubility	Soluble in water	
	Tricalcium Phosphate	
Form	Amorphous powder	
% Composition	Ca (38.76%), O (41.27%), and P (19.97%)	
Density	3.14	
Formula weight	310.17	
Solubility	Readily soluble in 3 N HCl and 2 $NHNO_3$; practically insoluble in water, alcohol, and acetic acid	
	Trimagnesium Phosphate	
% Composition	Mg (27.74%), O (48.69%), and P (23.57%)	
Formula weight	262.85	
	Trisodium Phosphate	
% Composition	Na (42.07%), O (39.04%), and P (18.89%)	Crystallizes with 8 and 12 mols H_2O
Formula weight	163.94	

Table 3. Current Frequency and Concentration of Use According to Duration and Type of Exposure.[19,20]

	Phosphoric Acid		Ammonium Phosphate		Diammonium Phosphate	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	489	0.000004-9	1	0.6	3	0.00046-5
Duration of Use						
Leave-On	168	0.000004-1.2	1	NR	NR	0.00046
Rinse off	311	0.00005-9	NR	0.6	3	5
Diluted for (bath) Use	10	0.34	NR	NR	NR	NR
Exposure Type						
Eye Area	7	NR	NR	NR	NR	NR
Incidental Ingestion	2	0.0003-0.21	NR	NR	NR	NR
Incidental Inhalation- Sprays	NR	0.001-0.26	NR	NR	NR	NR
Incidental Inhalation- Powders	NR	0.00001	NR	NR	NR	0.00046
Dermal Contact	154	0.000004-2.7	NR	NR	1	0.00046
Deodorant (underarm)	NR	0.068	NR	NR	NR	NR
Hair - Non-Coloring	123	0.00023-5.9	1	0.6	1	NR
Hair-Coloring	101	0.07-9	NR	NR	1	5
Nail	45	0.00049-0.1	NR	NR	NR	NR
Mucous Membrane	109	0.00005-0.34	NR	NR	NR	NR
Baby Products	NR	0.00001	NR	NR	NR	NR
	Disodium Phosphate		**Disodium Pyrophosphate**		**Pentasodium Triphosphate**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	280	0.000054-2.9	15	0.05-0.3	43	0.25-9.3
Duration of Use						
Leave-On	94	0.000054-1.2	NR	NR	3	0.25-9.3
Rinse off	176	0.000055-2.9	15	0.05-0.3	23	1.1
Diluted for (bath) Use	10	0.04-0.2	NR	NR	17	6-9
Exposure Type						
Eye Area	16	0.00095-1	NR	NR	NR	NR
Incidental Ingestion	1	0.5	8	NR	22	1.1
Incidental Inhalation- Sprays	NR	0.02-0.3	NR	NR	NR	NR
Incidental Inhalation- Powders	NR	0.00095-1.2**	NR	NR	NR	1**
Dermal Contact	132	0.000054-1.7	NR	NR	17	1-9
Deodorant (underarm)	NR	0.03	NR	NR	NR	NR
Hair - Non-Coloring	21	0.000055-2	NR	NR	NR	NR
Hair-Coloring	75	0.0025-2.9	7	0.05-0.3	NR	NR
Nail	NR	NR	NR	NR	NR	0.25-9.3
Mucous Membrane	48	0.04-0.5	8	NR	39	1.1-9
Baby Products	NR	NR	NR	NR	NR	NR
	Sodium Hexametaphosphate		**Sodium Metaphosphate**		**Sodium Phosphate**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	89	0.09-3	45	0.01-9.6	87	0.0000015-2.6
Duration of Use						
Leave-On	74	0.1-3	42	0.01-9.6	52	0.0000015-2
Rinse off	12	0.09-1.5	3	0.01-0.2	35	0.00007-2.6
Diluted for (bath) Use	3	NR	NR	0.03-0.5	NR	NR
Exposure Type						
Eye Area	19	NR	7	0.03-0.1	9	0.00007-0.5
Incidental Ingestion	2	NR	NR	0.14	7	0.25-0.55
Incidental Inhalation- Sprays	NR	NR	NR	0.04*	NR	0.000014
Incidental Inhalation- Powders	NR	0.1-3	NR	0.25	NR	0.00015-0.086
Dermal Contact	48	0.09-3	34	0.01-9.6	45	0.0000015-2.6
Deodorant (underarm)	NR	NR	NR	0.029	NR	0.099
Hair - Non-Coloring	NR	NR	NR	0.01-0.05	6	0.000014-2
Hair-Coloring	NR	NR	NR	0.15	1	NR
Nail	NR	NR	NR	0.03	NR	NR
Mucous Membrane	5	1.5	2	0.03-0.5	15	0.0032-2.6
Baby Products	NR	NR	NR	NR	1	0.00015

Table 3. Current Frequency and Concentration of Use According to Duration and Type of Exposure.[19,20]

	Sodium Phosphate, Monobasic		Tetrasodium Pyrophosphate		Trisodium Phosphate	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)

Totals/Conc. Range	31	NR	118	0.0000014-6	25	0.00045
Duration of Use						
Leave-On	11	NR	17	0.03-3	13	0.00045-7
Rinse off	20	NR	101	0.0000014-6	12	0.05-1.7
Diluted for (bath) Use	NR	NR	NR	NR	NR	0.08
Exposure Type						
Eye Area	1	NR	2	NR	5	0.00045
Incidental Ingestion	NR	NR	8	0.4-2	5	NR
Incidental Inhalation- Sprays	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Powders	NR	NR	NR	<0.1-1**	NR	7**
Dermal Contact	21	NR	42	0.0000014-3	9	0.00045-7
Deodorant (underarm)	NR	NR	NR	<0.01	NR	NR
Hair - Non-Coloring	1	NR	NR	0.000014-0.04	NR	0.25-0.5
Hair-Coloring	NR	NR	63	0.02-6	2	0.18-1.7
Nail	1	NR	1	0.03	6	3.7
Mucous Membrane	16	NR	8	0.0000014-2	6	0.08
Baby Products	NR	NR	NR	NR	NR	NR
	Dipotassium Phosphate		**Potassium Metaphosphate**		**Potassium Phosphate**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	31	0.002-1.8	NR	0.11	107	0.0000000014-0.9
Duration of Use						
Leave-On	10	0.002-0.3	NR	NR	61	0.0000000014-0.9
Rinse off	21	0.033-1.8	NR	0.11	45	0.00007-0.68
Diluted for (bath) Use	NR	NR	NR	NR	1	0.06
Exposure Type						
Eye Area	16	0.033-0.4	NR	NR	23	0.006-0.56
Incidental Ingestion	NR	NR	NR	NR	2	0.000007
Incidental Inhalation- Sprays	NR	NR	NR	NR	NR	0.00065-0.08
Incidental Inhalation- Powders	NR	0.002-0.3**	NR	NR	NR	0.0000000014-0.01**
Dermal Contact	27	0.002-0.47	NR	0.11	65	0.0000000014-0.9
Deodorant (underarm)	NR	NR	NR	NR	NR	0.07
Hair - Non-Coloring	1	NR	NR	NR	1	0.000003-0.6
Hair-Coloring	NR	1.8	NR	NR	4	0.2
Nail	NR	NR	NR	NR	1	NR
Mucous Membrane	1	0.05-0.066	NR	0.11	9	0.000007-0.06
Baby Products	NR	NR	NR	NR	NR	NR
	Tetrapotassium Pyrophosphate		**Calcium Dihydrogen Phosphate**		**Calcium Phosphate**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	95	NR	4	NR	NR	0.0001
Duration of Use						
Leave-On	5	NR	1	NR	NR	0.0001
Rinse off	90	NR	3	NR	NR	NR
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Exposure Type						
Eye Area	NR	NR	NR	NR	NR	NR
Incidental Ingestion	23	NR	3	NR	NR	NR
Incidental Inhalation- Sprays	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Powders	NR	NR	NR	NR	NR	NR
Dermal Contact	69	NR	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	1	NR	NR	0.0001
Mucous Membrane	23	NR	3	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

Table 3. Current Frequency and Concentration of Use According to Duration and Type of Exposure.[19,20]

	Calcium Pyrophosphate		Dicalcium Phosphate		Dicalcium PhosphateDihydrate	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	3	NR	327	0.000099-47.7	16	0.58-49
Duration of Use						
Leave-On	1	NR	322	0.04-10	11	0.58-6.8
Rinse off	2	NR	5	0.000099-47.7	5	49
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Exposure Type						
Eye Area	NR	NR	24	0.042-10	6	0.58
Incidental Ingestion	2	NR	222	0.3-47.7	9	6.8-49
Incidental Inhalation- Sprays	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Powders	NR	NR	14	0.04-2.2	NR	2.2
Dermal Contact	NR	NR	89	0.04-10	6	0.58-2.2
Deodorant (underarm)	NR	NR	NR	0.49	NR	NR
Hair - Non-Coloring	1	NR	NR	0.000099	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	2	NR	218	0.3-47.7	9	6.8-49
Baby Products	NR	NR	NR	NR	NR	NR
	Tricalcium Phosphate		**Trimagnesium Phosphate**			
	# of Uses	Conc. (%)	# of Uses	Conc. (%)		
Totals/Conc. Range	33	NR	1	NR		
Duration of Use						
Leave-On	31	NR	NR	NR		
Rinse off	2	NR	1	NR		
Diluted for (bath) Use	NR	NR	NR	NR		
Exposure Type						
Eye Area	NR	NR	NR	NR		
Incidental Ingestion	2	NR	1	NR		
Incidental Inhalation- Sprays	NR	NR	NR	NR		
Incidental Inhalation- Powders	26	0.099**-10	NR	NR		
Dermal Contact	30	NR	NR	NR		
Deodorant (underarm)	NR	0.4	NR	NR		
Hair - Non-Coloring	NR	NR	NR	NR		
Hair-Coloring	NR	NR	NR	NR		
Nail	NR	NR	NR	NR		
Mucous Membrane	2	NR	1	NR		
Baby Products	7	0.12	NR	NR		

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for (Bath) Use 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 a 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 4. Acute Inhalation Toxicity

Ingredient	Animals	Results
	Acids	
Phosphoric Acid (generated from pure red phosphorus ignited in an air stream). Target concentrations of smoke ranged from 111 to 6,731 mg/m^3 as Phosphoric Acid	New Zealand white rabbits (groups of 10), Porton strain rats (groups of 9 to 12), Porton strain mice (group of 20 or 50), and Dunkin-Hartley guinea pigs (groups of 10 or 20)	LC_{50}s (1-h exposure): 5337 mg/m^3 (rabbits), 3846 mg/m^3 (rats), 856 mg/m^3 (mice), and 193 mg/m^3 (guinea pigs). Lesions in larynx and trachea in all groups, except for guinea pigs.[67,70]
	Sodium Salts	
Disodium Pyrophosphate	Rats	LC_{50} (4-h exposure) > 0.58 mg/l air.[50]
Pentasodium Triphosphate	Rats	LC_{50} (4-h exposure) > 0.39 mg/l air.[50]
Sodium Polyphosphate/Sodium Hexametaphosphate	Rats	LC_{50} (4-h exposure) > 3.9 mg/l air.[50]
Sodium Phosphate	Rats	LC_{50} (4-h exposure) > 0.83 mg/l air.[50]
	Potassium Salts	
Tetrapotassium Pyrophosphate	Rats	LC_{50} (4-h exposure) > 1.1 mg/l air.[50]
	Calcium Salts	
Calcium Dihydrogen Phosphate	Rats (5 males and 5 females)	LC_{50} (4-h exposure) ≥ 2.6 mg/l air.[88,89]
Dicalcium Phosphate	Wistar rats (5 males and 5 females)	LC_{50} (4-h exposure) > 2.6 mg/l air.[69]

Table 5. Acute Oral Toxicity Studies

Ingredient	Test Concentration	Animals (number stated, if available from source)	Results
		Acids	
Phosphoric Acid	Not stated	Rats	LD_{50} = 1530 mg/kg.[3,70]
Phosphoric Acid	Not stated	Sprague-Dawley rats (12 females)	$LD_{50} \approx$ 2000 mg/kg.[70]
Phosphoric Acid	75%-85% solution	Rats	LD_{50} = 3160 mg/kg.[50]
Phosphoric Acid	85% solution	Rats	LD_{50} = 3380 mg/kg.[50]
Phosphoric Acid	85% solution	Sprague-Dawley albino rats (males and females)	LD_{50} = 3500 mg/kg.[70,71]
Phosphoric Acid	80% solution	Sprague-Dawley albino rats (males and females)	LD_{50} = 4200 mg/kg.[70,71]
Phosphoric Acid	75% solution	Sprague-Dawley albino rats (males and females)	LD_{50} = 4400 mg/kg.[70,71]
Phosphoric Acid		Rabbits	LD_{50} = 2740 mg/kg.[3]
		Ammonium Salts	
Ammonium Phosphate		Rats	LD_{50} >1000 mg/kg.[50]
Ammonium Phosphate		Rats	LD_{50} = 3250 mg/kg.[90]
Ammonium Phosphate		Rats	LD_{50} = 5750 mg/kg.[50]
Ammonium Phosphate		Rats	LD_{50} > 2000 mg/kg.[90]
Diammonium Phosphate		Rats	LD_{50} >1000 mg/kg.[50]
Diammonium Phosphate		Rats	LD_{50} > 2000 mg/kg.[90]
Diammonium Phosphate		Rats	LD_{50} = 6500 mg/kg.[50]
Diammonium Phosphate		Rats	LD_{50} > 25,100 mg/kg.[50]
		Sodium Salts	
Disodium Phosphate		Rats	LD_{50} = 5950 mg/kg.[50]
Disodium Pyrophosphate		Rats	LD_{50} >1000 mg/kg.[50]
Disodium Pyrophosphate		Rats	LD_{50} = 1690 mg/kg.[8]
Disodium Pyrophosphate		Rats	LD_{50} = 3600 mg/kg.[50]
Disodium Pyrophosphate		Rats	LD_{50} > 4000 mg/kg.[50,91]
Disodium Pyrophosphate		Mice	LD_{50} = 3350 mg/kg.[8]
Disodium Pyrophosphate		Hamsters	LD_{50} = 1660 mg/kg.[8]
Pentasodium Triphosphate		Rats	LD_{50} = 1700 mg/kg.[8]
Pentasodium Triphosphate		Rats	LD_{50} = 5010 mg/kg.[50]
Pentasodium Triphosphate		Mice	LD_{50} = 2380 mg/kg.[8]
Pentasodium Triphosphate		Rabbits	LD_{50} = 2500 mg/kg.[8]
Sodium Hexametaphosphate		Rats	LD_{50} = 2400 mg/kg.[8]
Sodium Hexametaphosphate		Mice	LD_{50} = 3700 mg/kg.[8]
Sodium Polyphosphate/Sodium Hexametaphosphate		Rats	LD_{50} = 2400 mg/kg.[50]
Sodium Polyphosphate/Sodium Hexametaphosphate		Rats	LD_{50} = 2900 mg/kg.[50,92]

Table 5. Acute Oral Toxicity Studies

Ingredient	Test Concentration	Animals (number stated, if available from source)	Results
Sodium Polyphosphate/Sodium Hexametaphosphate		Rats	LD_{50} >10,000 mg/kg.[50]
Sodium Polyphosphate/Sodium Hexametaphosphate		Mice	LD_{50} = 3700 mg/kg.[8]
Sodium Phosphate		Rats	LD_{50} = 4100 mg/kg.[8]
Sodium Phosphate		Rats	LD_{50} = 7100 mg/kg.[50]
Sodium Phosphate		Rats	LD_{50} = 8390 mg/kg.[50,68]
Sodium Phosphate		Mice	LD_{50} > 3700 mg/kg.[50,8]
Sodium Phosphate		Guinea pigs	LD_{50} > 2000 mg/kg.[50,91]
Sodium Trimetaphosphate		Rats	LD_{50} = 10600 mg/kg.[50]
Tetrasodium Pyrophosphate		Rats	LD_{50} = 1380 mg/kg.[8]
Tetrasodium Pyrophosphate		Rats (female)	LD_{50} = 1825 mg/kg.[50]
Tetrasodium Pyrophosphate		Rats (male)	LD_{50} = 2150 mg/kg.[50]
Tetrasodium Pyrophosphate		Rats	LD_{50} = 3770 mg/kg.[50]
Tetrasodium Pyrophosphate		Rats	LD_{50} = 1380 mg/kg.[8]
Tetrasodium Pyrophosphate (~ 67%) and Potassium Metaphosphate (~ 33%)		Rats	LD_{50} = 4000 mg/kg.[8]
Tetrasodium Pyrophosphate	200 mg/ml suspension in distilled water	Sprague-Dawley rats (females, groups of 5)	No clinical signs or necropsy findings. LD_{50} > 2000 mg/kg.[32,93]
Tetrasodium Pyrophosphate		Mice	LD_{50} = 1300 mg/kg.[8]
Trisodium Phosphate		Rats	LD_{50} > 2000 mg/kg.[50,94]
Trisodium Phosphate		Rats	LD_{50} = 4100 mg/kg.[50]
Trisodium Phosphate		Rats	LD_{50} = 4150 mg/kg.[50]
Trisodium Phosphate		Rats (female)	LD_{50} < 5000 mg/kg.[50]
Trisodium Phosphate	20% solution	Rats	LD_{50} = 6500 mg/kg.[50,95]
Trisodium Phosphate		Rats	LD_{50} = 7800 mg/kg.[50]
		Potassium Salts	
Dipotassium Phosphate		Rats	LD_{50} > 500 mg/kg.[50]
Dipotassium Phosphate		Rats	LD_{50} > 1000 mg/kg.[50]
Dipotassium Phosphate(liquid)		Rats	LD_{50} = 4810 mg/kg.[50]
Dipotassium Phosphate		Rats	LD_{50} = 5700 mg/kg.[50]
Tetrapotassium Pyrophosphate		Rats (male)	LD_{50} > 1000 mg/kg.[50]
Tetrapotassium Pyrophosphate		Rats	LD_{50} = 2980 mg/kg.[50]
Tetrapotassium Pyrophosphate		Rats	LD_{50} = 3160 mg/kg.[50]

Table 5. Acute Oral Toxicity Studies

Ingredient	Test Concentration	Animals (number stated, if available from source)	Results
Tetrapotassium Pyrophosphate		Rats	LD_{50} = 3550 mg/kg.[50]
Tetrapotassium Pyrophosphate	Solution (concentration not stated)	Rats	LD_{50} = 2440 mg/kg.[50]
Tetrapotassium Pyrophosphate	Solution (concentration not stated)	Rats	LD_{50} < 5000 mg/kg.[50]
Tetrapotassium Pyrophosphate		Mice	LD_{50} = 1000 mg/kg.[50,96]
Dipotassium Phosphate		Mice	LD_{50} = 1700 mg/kg.[50,97]
Pentapotassium Triphosphate		Rats (male)	LD_{50} > 1000 mg/kg.[50]
Potassium Phosphate		Rats (male)	LD_{50} > 4640 mg/kg.[50]
Potassium Phosphate		Rats	LD_{50} = 7100 mg/kg.[50]
Potassium Phosphate		Rats	LD_{50} = 2820 mg/kg.[8]
Potassium Phosphate		Mice	LD_{50} = 1700 mg/kg.[50,98]
Potassium Phosphate		Mice	LD_{50} ≈ 3200 mg/kg.[8]
		Calcium Salts	
Calcium Dihydrogen Phosphate (in distilled water)		Female Sprague-Dawley rats (groups of 3)	LD_{50} > 2000 mg/kg.[88,89]
Calcium Dihydrogen Phosphate		Female Sprague-Dawley rats (groups of 5)	LD_{50} > 10000 mg/kg.[88,89]
Calcium Dihydrogen Phosphate		Albino rabbits (5 males and 5 females)	LD_{50} > 2000 mg/kg.[89]
Calcium Phosphate		Rats	LD_{50} > 1000 mg/kg.[50]
Calcium Phosphate		Rats	LD_{50} = 2170 mg/kg.[50]
Calcium Phosphate		Rats (female)	LD_{50} = 3986 mg/kg.[50]
Calcium Phosphate		Rats (male)	LD_{50} > 5000 mg/kg.[50]
Calcium Phosphate		Mice	LD_{50} = 4600 mg/kg.[8]
Dicalcium Phosphate		6 Sprague-Dawley rats (female)	LD_{50} ≥ 2000 mg/kg.[69]
Dicalcium Phosphate		Rats	LD_{50} = 7100 mg/kg.[50]
Dicalcium Phosphate		Rats	LD_{50} > 7940 mg/kg.[50]
Dicalcium Phosphate		10 Sprague-Dawley rats (female)	LD_{50} > 10000 mg/kg.[69]
Dicalcium Phosphate		Mice	LD_{50} ≈ 1700 mg/kg.[18]
Tricalcium Phosphate		Rats	LD_{50} > 5000 mg/kg.[50]
Tricalcium Phosphate		Sprague-Dawley rats (female, groups of 3)	LD_{50} > 2000 mg/kg.[57]
Calcium Pyrophosphate		Rats	LD_{50} > 10,000 mg/kg.[50]

Table 5. Acute Oral Toxicity Studies

Ingredient	Test Concentration	Animals (number stated, if available from source)	Results
Magnesium Salts			
Magnesium Phosphate		Rats	LD_{50} > 1000 mg/kg.[50]
Magnesium Phosphate		Rats	LD_{50} > 4640 mg/kg.[50]
Magnesium Phosphate		Rats	LD_{50} > 5000 mg/kg.[50]
Trimagnesium Phosphate		Rats	LD_{50} > 10000 mg/kg.[50]
Magnesium Phosphate	Solution (concentration not stated)	Rabbits	LD_{50} > 2000 mg/kg.[50]

Table 6. Acute Dermal Toxicity Studies

Ingredient	Test Concentration/Dose	Animals (number stated, if available from source)	Results
		Acids	
Phosphoric Acid	85% solution	New Zealand white rabbits (males and females; groups of up to 2)	LD_{50} > 1260 mg/kg.[70]
Phosphoric Acid		Rabbits	LD_{50} = 2740 mg/kg.[50,68]
Phosphoric Acid	75% and 80% solutions	New Zealand white rabbits (males and females; groups of up to 2)	LD_{50} > 3160 mg/kg.[70]
Phosphoric Acid	85% solution	Rabbits	LD_{50} > 2000 mg/kg.[50]
		Ammonium Salts	
Ammonium Phosphate		Rats	LD_{50} > 5000 mg/kg.[90]
Ammonium Phosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]
Diammonium Phosphate		Rats	LD_{50} > 5000 mg/kg.[90]
Diammonium Phosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]
Diammonium Phosphate		Rabbits	LD_{50} > 10,000 mg/kg.[50]
Diammonium Phosphate		Rats	LD_{50} > 5000 mg/kg.[99]
		Sodium Salts	
Disodium Phosphate		Rabbits	LD_{50} >7940 mg/kg.[50]
Disodium Pyrophosphate		Rabbits	LD_{50} > 300 mg/kg.[100]
Disodium Pyrophosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]
Pentasodium Triphosphate		Rabbits	LD_{50} = 4640 mg/kg.[50]
Pentasodium Triphosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]
Sodium Phosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]
Sodium Polyphosphate/Sodium Hexametaphosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]
Tetrasodium Pyrophosphate		20 Sprague-Dawley rats	No clinical signs or necropsy findings. LD_{50} > 2000 mg/kg.[33]
Tetrasodium Pyrophosphate		Rabbits	LD_{50} > 2000 mg/kg.[50]
Tetrasodium Pyrophosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]
Trisodium Phosphate		Rabbits	LD_{50} > 300 mg/kg.[100]
Trisodium Phosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]
		Potassium Salts	
Dipotassium Phosphate		Rabbits	LD_{50} > 300 mg/kg.[50]
Dipotassium Phosphate(liquid)		Rabbits	LD_{50} > 5000 mg/kg.[50]
Dipotassium Phosphate		Rabbits	LD_{50} > 5000 mg/kg.[50]
Pentapotassium Triphosphate		Rabbits	LD_{50} > 4640 mg/kg.[50]
Potassium Phosphate		Rabbits	LD_{50} > 4640 mg/kg.[50]
Potassium Phosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]
Tetrapotassium Pyrophosphate		Rabbits	LD_{50} > 2000 mg/kg.[50]

Table 6. Acute Dermal Toxicity Studies

Ingredient	Test Concentration/Dose	Animals (number stated, if available from source)	Results
Tetrapotassium Pyrophosphate		Rabbits	LD_{50} > 4640 mg/kg.[50]
Tetrapotassium Pyrophosphate (liquid)		Rabbits	LD_{50} > 5000 mg/kg.[50]
Tetrapotassium Pyrophosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]
Tetrapotassium Pyrophosphate		Rabbits	LD_{50} > 10,000 mg/kg.[50]
Calcium Salts			
Calcium Dihydrogen Phosphate	2000 mg/kg	Rabbits (5 males and 5 females)	Severe erythema and mild edema. LD_{50} > 2000 mg/kg.[88]
Calcium Phosphate		Rabbits	LD_{50} > 300 mg/kg.[100]
Calcium Phosphate		Rabbits	LD_{50} > 2000 mg/kg.[50]
Calcium Pyrophosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]
Dicalcium Phosphate	2000 mg/kg	Stauffland albino rabbits (5 males and 5 females)	LD_{50} > 2000 mg/kg.[69]
Dicalcium Phosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]
Tricalcium Phosphate		Rabbits	LD_{50} > 2000 mg/kg.[50]
Magnesium Salts			
Magnesium Phosphate	Solution (concentration not stated)	Rabbits	LD_{50} > 2000 mg/kg.[50]
Magnesium Phosphate		Rabbits	LD_{50} > 4640 mg/kg.[50]
Trimagnesium Phosphate		Rabbits	LD_{50} > 7940 mg/kg.[50]

Table 7. Repeated Dose Oral Toxicity Studies

Ingredient	Test Concentration/Dose	Animals (number stated, if available from source)	Results
		Acids	
Phosphoric Acid	Oral doses (by gavage) of 0, 125, 250, or 500 mg/kg/day for 42 days (males) and 40 to 42 days (females)	Sprague-Dawley rats (13/sex/dose)	2 females of 500 mg/kg/day group died. NOAEL = 250 mg/kg/day.[67,70]
Phosphoric Acid	up to 0.75% in diet (338 mg/kg/day*) for > 52 weeks	Rats	NOEL = 338 mg/kg/day*. [50,91]
Phosphoric Acid	0, 35, 105, or 211 mg/kg/day for 70 days	Sheep	NOAEL = 105 mg/kg/day.[101]
		Ammonium Salts	
Diammonium Phosphate	Oral doses (by gavage) of 0, 250, 750, or 1500 mg/kg/day (7 days/week) for 35 days	Rats (groups of 10 [5 males and 5 females/group])	Histological examination of stomach revealed submucosal inflammation (not dose-dependent) at all doses. NOAEL = 250 mg/kg/day.[90,102]
Diammonium Phosphate	Increasing oral doses (in drinking water) of 300 to 700 mg/kg/day for 5 to 16 months	10 Rabbits (females)	Average weight of parathyroid glands (only parameter assessed) was 235% of control values.[99]
		Sodium Salts	
Disodium Phosphate	10% in diet for 24 h to 72 h	Rats	Histological and histochemical changes in the kidneys.[8,103]
Disodium Phosphate	1.8%, 3%, and 5% in modified Sherman diet for 6 months	Young rats (groups of 34 to 36)	Significant decrease in growth and kidney damage (nephrocalcinosis) at dietary concentrations of 3% and 5%. Normal growth and slight increase (statistically significant) in kidney weight at 1.8% in the diet.[8,104]
Disodium Phosphate	0%, 1.1%, 1.8%, 3%, or 5% in diet (0, 495, 810, 1350, and 2250 mg/kg/day*) for 39 weeks	Rats	Slight kidney calcification. LOEL = 495 mg/kg/day*.[8,50,105]
Disodium Phosphate	1%, 2.5%, and 5% in Sherman diet for 16 weeks	Rats	Severe kidney damage in 5% dietary group (number of animals not stated). Hypertrophy and hemorrhage of the stomach (number of animals not stated).[8,106]
Disodium Phosphate	5% Disodium Phosphatein the diet for 1 month (2571 mg/kg/day)	Weanling rats	Renal tubular necrosis. LOEL < 2571 mg/kg/day [assuming that 0.35 kg rat consumes18 g food/day][32,92]
Disodium Phosphate	1%, 2.5%, or 5% in diet containing 0.6% calcium and 0.5% phosphorus for 100 days	20 rats per sex	Renal histopathology, decreased renal function, and increased kidney weight in all dietary groups. LOEL for 5% in diet = 2571 mg/kg/day (assuming that 0.35-kg rat consumed 18 g food per day).[32,106]

Table 7. Repeated Dose Oral Toxicity Studies

Ingredient	Test Concentration/Dose	Animals (number stated, if available from source)	Results
Disodium Pyrophosphate	1%, 2.5%, or 5% in basal diet (contained 0.6% calcium and 0.5% phosphorus) for 100 days	Groups of 20 rats per sex	Renal histopathology, decreased renal function, and increased kidney weight in all groups except 1% dietary group. LOEL for 1% dietary group = 450 mg/kg/day (assuming that 0.35 kg rat consumes18 g food/day).[32,106]
Pentasodium Triphosphate	0%, 0.2%, 2%, or 10% (0, 103, 900 and 5143 mg/kg/day*) for 30 days	Rats	NOEL = 103 mg/kg/day. [Extrapolated from level of chemical in diet, assuming 0.35-kg rat eats 18 g food/day.][92]
Pentasodium Triphosphate	0%, 0.2%, 2%, or 10% (0, 90, 900 and 4500 mg/kg/day*) for 30 days	Rats	NOEL = 90 mg/kg/day [Extrapolated from level of chemical in diet, assuming 0.35-kg rat eats 18 g food/day.][92]
Pentasodium Triphosphate	1% solutions (pH of 5) of 3%, and 5% Pentasodium Triphosphate (effective concentrations of 0.03% and 0.05% [14 and 23 mg/kg/day]*, respectively) in Sherman diet for 24 weeks	Groups of rats (36 males, 36 females/group)	Growth retardation at 0.05% in diet. Temporary growth retardation at 0.03% in diet. Nephrocalcinosis at both concentrations.[8,107,108,104]
Pentasodium Triphosphate	0%, 1.1%, 1.8%, 3%, and 5% (0, 495, 810, 1350, and 2250 mg/kg/day*) for 39 weeks	Rats	LOEL = 495 mg/kg/day*.[50,105]
Pentasodium Triphosphate	1.8%, 3%, and 5% (pH of 5 for each) (810, 1350, 2250 mg/kg/day*) in Sherman diet for 24 weeks	Groups of rats (36 males, 36 females/group)	Growth retardation at 5% in diet, temporary growth retardation at 3% in diet, and normal growth at 1.8% in diet. Nephrocalcinosis at 1.8%, 3%, or 5% in diet. Extent of kidney damage less at test substance pH of 5 than at pH 9.5.[8,107,108,104]
Pentasodium Triphosphate	0.05%, 0.5%, or 5% in diet (23, 225, 2250 mg/kg/day*) for 2 years	Weanling rats (groups of 50 males and 50 females)	Growth reduction only at 5% in diet (significant in males; slight in females). Smaller number (not stated) of rats fed 5% in diet survived. Low grade of anemia and increased kidney weight only at 5% in diet. NOEL = 225 mg/kg/day*.[8,92]
Pentasodium Triphosphate	Oral dose rate of 0.1 g/kg/day for 1 month (1 dog). 2 other dogs dosed similarly for 1 month, and dose had increased to 4 g/kg/day by end of 5-month period	3 dogs	Kidney tubule damage in dogs receiving higher doses. No treatment-related changes in dog dosed with 0.1 g/kg/day only.[8]
Pentasodium Triphosphate	0 and 100 mg/kg/day for 30 days	Dogs	NOAEL = 100 mg/kg/day.[92]

Table 7. Repeated Dose Oral Toxicity Studies

Ingredient	Test Concentration/Dose	Animals (number stated, if available from source)	Results
Sodium Hexametaphosphate	0.9% and 35% in diet for up to 150 days. Control group: diet containing 0.4% P and 0.5% Ca	Groups of 12 male rats	Kidney weight significantly heavier in 30% dietary group (possibly due to high salt load on kidneys), when compared to control. No histopathological abnormalities in either group.[8,109]
Sodium Hexametaphosphate	0.2%, 2%, or 10% in diet for 1 month	Groups of 5 weanling male rats	Increased relative kidney weight and renal tubular necrosis at 120% in diet. Dietary no-effect-level of 0.2% in diet (equivalent to 103 mg/kg/day, assuming that 0.35-kg rat consumes 18 g food/day).[32,92]
Sodium Hexametaphosphate	0.05%, 0.5%, or 5% in diet(23, 225, 2250 mg/kg/day*) for 2 years	Groups of 50 male and 50 female weanling rats	Calcification and increased kidney weight (not significant changes) in 5% dietary group. High mortality in all groups (unrelated to dietary concentration).[8]
Sodium Hexametaphosphate	1% in diet (450 mg/kg/day*) containing iron (1000 ppm) and iodine (30 ppm) for 9 months. Control group: unfortified salt diet	8 Wistar/NIN rats	No gross bone abnormality. Normal histology of kidneys and parathyroid gland in test and control groups.[110]
Sodium Hexametaphosphate	Oral dose rate of 0.1 g/kg/day for 1 month (1 dog). 2 other dogs dosed similarly for 1 month, and dose had increased to 4 g/kg/day by end of 5-month period	3 dogs	Kidney tubule damage in dogs receiving higher doses. No treatment-related changes in dog dosed with 0.1 g/kg/day only.[8]
Sodium Polyphosphate/Sodium Hexametaphosphate	0%, 0.2%, 2%, or 10% in diet for 30 days	Rats	NOEL = 103 mg/kg/day*.[92]
Sodium Polyphosphate/Sodium Hexametaphosphate	0%, 0.1%, 1%, and 10% in diet (0, 45, 450, 4500 mg/kg/day*) for 104 weeks	Rats	NOAEL = 450 mg/kg/day*.[92]
Sodium Polyphosphate/Sodium Hexametaphosphate	0%, 0.05%, 0.5%, or 5% in diet (0, 23, 225, 2250 mg/kg/day*) for 104 weeks	Rats	NOAEL = 225 mg/kg/day*.[92]
Sodium Polyphosphate/Sodium Hexametaphosphate	0%, 0.93%, or 3.5% in diet (0, 419, 1575 mg/kg/day*) for 21 weeks	Rats	NOAEL = 1575 mg/kg/day*.[50,109]
Sodium Polyphosphate/Sodium Hexametaphosphate	0 or 100 mg/kg/day for 30 days	Dogs	NOAEL = 100 mg/kg/day.[92]
Sodium Phosphate	0.4% or 0.6% in diet for 28 days	Juvenile female Wistar rats (RIV:TOX)	At 0.6% in diet, significant increase in kidney weight (25%) and in incidence of nephrocalcinosis.[32,111]

Table 7. Repeated Dose Oral Toxicity Studies

Ingredient	Test Concentration/Dose	Animals (number stated, if available from source)	Results
Sodium Phosphate	1%, 2.5%, or 5% in Sherman diet for 16 weeks	Groups of 20 male and female rats	Increased kidney weight (females) and decreased kidney function (males) at ≥ 2.5% in diet. Kidney damage (calcification, degeneration, and necrosis) in greater % of rats in 1% dietary group, when compared to control group.[8]
Sodium Phosphate	1.8%, 3%, or 5% in modified Sherman diet (810, 1350, 2250 mg/kg/day*) for 6 months	Groups of 34 to 36 young rats	Nephrocalcinosis in 3% and 5% dietary groups. At microscopic examination, kidney calcification in some of the animals (number not stated). Slight increase (statistically significant) in kidney weight in 1.8% dietary group.
Sodium Phosphate	8% in diet for 7 months or until exitus	Weanling rats	Gradual bone decalcification, renal calcium deposition, and significant parathyroid hypertrophy and hyperplasia. Histological evidence of metastatic calcium deposits in renal tubules and long-bone periosteum and endosteum.[61]
Sodium Phosphate	1.1% in diet (495 mg/kg/day*) for 39 weeks	Rats	Slight degree of kidney calcification.[8,105]
Sodium Phosphate	0, 43, 129, or 258 mg/kg/day for 70 days	Sheep	NOEL = 258 mg/kg/day.[101]
Sodium Trimetaphosphate	0.2%, 2%, or 10% in diet for 1 month	Weanling male rats (5 per group)	Reduced body weight, increased relative kidney weights, and renal tubular necrosis at 10% in diet. Acute inflammation or pelvic lesions in some of the rats (number not stated) fed 2% in diet. Dietary no-effect-level of 0.2% in diet (equivalent to 103 mg/kg/day, assuming that 0.35-kg rat consumes 18 g of food/day).[32,92]
Sodium Trimetaphosphate	0.1%, 1%, or 10% in diet (45, 450, 4500 mg/kg/day*) for 2 years	Rats	At 10% in diet, substantial growth retardation (males and females) and anemia (females).[112]
Sodium Trimetaphosphate	0.05%, 0.5%, or 5% in diet (23, 225, 2250 mg/kg/day*) for 2 years	Rats	Substantial growth retardation in males of 5% dietary group, but females slightly affected. 65% of rats examined in 5% dietary group presented with intertubular calcification, as distinguished from the coexistent pyelonephritis present in old rats. NOAEL = 450 mg/kg/day [Extrapolated from level of chemical in diet, assuming 0.35-kg rat eats 18 g food/day.][92,112]

Table 7. Repeated Dose Oral Toxicity Studies

Ingredient	Test Concentration/Dose	Animals (number stated, if available from source)	Results
Sodium Trimetaphosphate	0 and 100 mg/kg/day for 30 days	Dogs	NOAEL = 100 mg/kg/day.[92]
Tetrasodium Pyrophosphate	250, 500, or 1000 mg/kg/day by gavage for 90 days (5 days/week) (OECD Guideline 408)	Groups of 20 Sprague-Dawley rats (10 males and 10 females/group)	No treatment-related mortalities. Increased white blood cell count (males and females) and decreased red blood cell count (males) at 1000 mg/kg/day. Significantly increased liver weight in males and females of 500 and 1000 mg/kg/day groups. Kidney lesions in males and females of 1000 mg/kg/day group. NOEL = 250 mg/kg/day; NOAEL = 500 mg/kg/day.[33]
Tetrasodium Pyrophosphate	0%, 1.1%, 1.8%, 3%, or 5% in diet (0, 495, 810, 1350 mg/kg/day*) for 39 weeks	Rats	LOEL = 495 mg/kg/day*.[50,105]
Trisodium Phosphate	8% in diet (3600 mg/kg/day*) for 7 months or until animals died	Mature rats	Pathological effects in parathyroids, kidneys, and bones. LOEL < 3600 mg/kg/day*. [8,113,114]
		Sodium and Potassium Salts	
Diets high (1.5%) in P (as monophosphate or tripolyphosphate sodium or potassium salts)	Feeding for 13 days	Male rats	Nephrocalcinosis.[32,115]
Tetrasodium Pyrophosphate + Potassium Metaphosphate	0.5% commercial preparation containing 67% Tetrasodium Pyrophosphate and 33% Potassium Metaphosphate (effective concentration [Tetrasodium Pyrophosphate = 0.5% x 67% = 0.34%; effective concentration [Potassium Metaphosphate] = 0.5% x 33% = 0.17%]	Rats (10 males, 10 females). Feeding continued through 2nd and 3rd generations	Growth, average lifespan, and kidney weight normal.[8,60]
Tetrasodium Pyrophosphate + Potassium Metaphosphate	1% commercial preparation containing 67% Tetrasodium Pyrophosphate and 33%Potassium Metaphosphate (effective concentration [Tetrasodium Pyrophosphate = 1% x 67% = 0.67%; effective concentration [Potassium Metaphosphate] = 1% x 33% = 0.33 %]	Rats (10 males, 10 females). Feeding continued through 2nd and 3rd generations	Growth and average lifespan normal. Nephrocalcinosis and slight increase (significant increase only in males) in kidney weight observed.[8,60]

Table 7. Repeated Dose Oral Toxicity Studies

Ingredient	Test Concentration/Dose	Animals (number stated, if available from source)	Results
Tetrasodium Pyrophosphate + Potassium Metaphosphate	2.5% commercial preparation containing 67% Tetrasodium Pyrophosphate and 33% Potassium Metaphosphate (effective concentration [Tetrasodium Pyrophosphate = 2.5% x 67% = 1.7%; effective concentration [Potassium Metaphosphate] =2.5% x 33% = 0.83%]	Rats (10 males, 10 females). Feeding continued through 2nd and 3rd generations	Growth and average lifespan normal. Nephrocalcinosis and increased kidney weight observed.[8,60]
Tetrasodium Pyrophosphate + Potassium Metaphosphate	5% commercial preparation containing 67% Tetrasodium Pyrophosphate and 33% Potassium Metaphosphate (effective concentration [Tetrasodium Pyrophosphate = 5% x 67% = 3.4%; effective concentration [Potassium Metaphosphate] = 5% x 33% = 1.7%]	Rats (10 males, 10 females). Feeding continued through 2nd and 3rd generations	Growth retardation, increased kidney weight, and nephrocalcinosis observed.[8,60]
	Potassium Salts		
Dipotassium Phosphate	10% in diet for 8 weeks	Male Wistar rats	Nephrotoxicity at 10% in diet.[64]
Dipotassium Phosphate	0.87% and 5.1% in diet for 60 days and 150 days. 5.1% in diet equivalent to 2623 mg/kg/day*	Groups of 12 Wistar male rats	Kidney weight significantly increased after 150 days of feeding at 5.1% in diet; no histopathological lesions in kidney. No other treatment-related effects at gross or histopathological examination. NOAEL = 2623 mg/kg/day*.[18]
Dipotassium Phosphate	0.87% or 5.1% for 21 weeks	Rats	NOAEL = 2295 mg/kg/day*.[50,109]
Dipotassium Phosphate	5% in diet (2250 mg/kg/day*) in medium-term bioassay	Male Wistar rats	Renal calcification and severe nephropathy.[64]
Dipotassium Phosphate	Oral doses (by gavage) of 1000 mg/kg/day for 42 days (males) and 42 to 54 days (females)	Rats (males and females)	Significant decreases in liver and heart weights-to-body weight ratio. No gross or histopathological alterations. LOEL = 1000 mg/kg/day.[116]
Dipotassium Phosphate	Oral doses (by gavage) of 1000 mg/kg/day for 42 days (males) and for 42 to 54 days (females)	Sprague-Dawley rats (16 males and 16 females/group)	No deaths or abnormal clinical changes. Statistically significant reductions in red blood cells in females, but not in males. Significantly lower relative liver and heart weights observed not considered toxicological findings, due to absence of histopathological changes. LOEL = 1000 mg/kg/day.[18]

Table 7. Repeated Dose Oral Toxicity Studies

Ingredient	Test Concentration/Dose	Animals (number stated, if available from source)	Results
Dipotassium Phosphate	Diet providing 800 mg/kg/day for 14 and 38 weeks	15 Beagle dogs	Renal damage consisted of disseminated tubular atrophy (usually of the proximal tubules), focal scar tissue, and nephrocalcinosis. Renal morphological changes in all dogs after 14 and 38 weeks; renal damage greater after 38 weeks. LOEL < 800 mg/kg/day. [18, 50,117,118]
Tetrapotassium Pyrophosphate	0.6%, 1.25%, 2.5%, 5%, or 10% in diet (270, 563, 2250, 4500 mg/kg/day*) (to estimate maximum tolerable dose for long-term carcinogenicity study)	Groups of 60 male and female F344 rats	3 rats (from 10% dietary group) died of renal failure. Histopathological exam results for 5% and 10% dietary groups: necrosis and calcification of renal tubules, ulceration and /or granuloma formation in tongue mucosa, and hypertrophy of salivary glands.[119]
		Calcium Salts	
Calcium Phosphate	0.8% calcium and 0.9% phosphorus in diet (duration not stated)	Guinea pigs	Calcium deposits in soft tissues. Reduction in deposits when phosphorus content reduced to 0.5%.[8,120]
Calcium Phosphate	0.56% calcium and 0.42% phosphorus in the diet for up to 150 days	12 rats	No adverse physiological effects at necropsy or microscopic examination.[8,109]
Calcium Phosphate	0.47% calcium and 0.43% phosphorus in the diet for up to 150 days	12 rats	No adverse physiological effects at necropsy or microscopic examination.[8,109]
Calcium Phosphate	0.5% calcium and 1.30% phosphorus in the diet for up to 150 days	12 rats	No adverse physiological effects at necropsy or microscopic examination.[8,109]
Calcium Phosphate	High phosphorus containing diets (Ca:P ratios of up to 1:4) for 88 months	Cinnamon ringtail monkeys (*Cebus albiffrons*)	Minor bone changes observed microscopically.[8,121]
Calcium Pyrophosphate (in saline; β-)	Feeding 7 days/week (30 mg/kg/day) for 90 days.	Sprague-Dawley rats (10 males, 10 females)	No deaths or adverse toxic effects.[39]
Dicalcium Phosphate	Doses of 0, 250, 500, or 1000 mg/kg/day by gavage for 28 days	Rats (10 per sex in control and highest dose groups; 5 per sex in other groups)	No treatment-related clinical, hematological, or necropsy findings. Statistically significant increase in relative liver weight in males of the 250 mg/kg group, but no morphological findings in the liver. NOAEL = 1000 mg/kg/day.[88,122]
Dicalcium Phosphate	Doses of 0, 250, 500, or 1000 mg/kg/day for 28 days by gastric intubation	Sprague-Dawley rats (10/sex/dose)	No gross or microscopic effects. NOAEL > 1000 mg/kg/day.[69]

Table 7. Repeated Dose Oral Toxicity Studies

Ingredient	Test Concentration/Dose	Animals (number stated, if available from source)	Results
Tricalcium Phosphate	Doses of 0, 250, 500, or 1000 mg/kg/day by gavage. Males dosed from 2 weeks before mating to end of mating. Females dosed from 2 weeks before mating to day 4 of lactation (including the mating and gestation periods)	Rats (10 per sex in each group)	No deaths or toxicologically significant findings. NOAEL = 1000 mg/kg/day.[56,57]

NOEL = no-observed-effect level; NOAEL = no-observed-adverse-effect level; LOEL = lowest-observed-effect level
*Extrapolated from level of chemical in diet, assuming 0.4 kg rat eats 18 g food/day.

Table 8. Reproductive and Developmental Toxicity Studies

Ingredient	Test Protocol	Animals/Embryos	Results
		Acids	
Phosphoric Acid	0.4% and 0.75% in diet for 90 weeks	Rats from 3 successive generations (number not stated)	No adverse effects on reproduction at either dietary concentration.[8,123]
Phosphoric Acid	Oral doses of 0, 125, 250, or 500 mg/kg/day, to male rats for 42 days (2 weeks prior to mating to 2 weeks after mating); to female rats for 40 to 52 days (2 weeks prior to mating to day 4 post partum)	Rats (13 males 13 females/group)	No reproductive effects or treatment-related changes in neonatal survival or external abnormalities.[67,70]
		Ammonium Salts	
Diammonium Phosphate	Oral doses of 0, 250, 750, or 1500 mg/kg/day (7 days/week) for 35 days	Rats (5 males and 10 females/group)	No reproductive or developmental effects at doses administered. NOAEL = 1500 mg/kg/day.[90,102]
		Sodium Salts	
Disodium Pyrophosphate	Doses (in water) up to 335 mg/kg/day on gestation days 6-15	19 to 22 CD-1 mice	No treatment-related effects (NOEL > 335 mg/kg).[124]
Disodium Pyrophosphate	Doses (in water) up to 169 mg/kg/day on gestation days 6-15	21 to 24 Wistar rats	No treatment-related effects (NOEL > 169 mg/kg).[124]
Disodium Pyrophosphate	Doses (in water) up to 166 mg/kg/day on gestation days 6-10	20 to 22 Golden hamsters	No treatment-related effects (NOEL > 166 mg/kg).[124]
Disodium Pyrophosphate	Doses (in water) up to 128 mg/kg/day on gestation days 6-18	9 to 12 Dutch-belted rabbits	No treatment-related effects (NOEL > 128 mg/kg).[124]
Pentasodium Triphosphate	Oral doses (in water) up to 238 mg/kg/day on gestation days 6-15	Groups of 21 to 24 pregnant albino, CD-1 outbred mice.	No clearly discernible treatment-related effect on nidation or on maternal or fetal survival. Number of abnormalities (in soft or skeletal tissues) in test animals did not differ from number occurring in sham-treated controls. NOEL > 238 mg/kg.[125,126]
Pentasodium Triphosphate	Oral doses (in water) up to 170 mg/kg/day on gestation days 6-15	Groups of 19 to 23 Wistar albino rats	No clearly discernible treatment-related effect on nidation or on maternal or fetal survival. Number of abnormalities (in soft or skeletal tissues) in test animals did not differ from number occurring in sham-treated controls. NOEL > 170 mg/kg.[125,126]
Pentasodium Triphosphate	Oral doses (in water) up to 141 mg/kg/day on gestation days 6-10	Groups of 20 to 21 pregnant female golden hamsters	No clearly discernible treatment-related effect on nidation or on maternal or fetal survival. Number of abnormalities (in soft or skeletal tissues) in test animals did not differ from number occurring in sham-treated controls. NOEL > 141 mg/kg.[125,126]

Table 8. Reproductive and Developmental Toxicity Studies

Ingredient	Test Protocol	Animals/Embryos	Results
Pentasodium Triphosphate	Oral doses (in water) up to 250 mg/kg/day on gestation days 6-18	Groups of 13 to 16 pregnant female Dutch-belted rabbits	No clearly discernible treatment-related effect on nidation or on maternal or fetal survival. Number of abnormalities (in soft or skeletal tissues) in test animals did not differ from number occurring in sham-treated controls. NOEL > 250 mg/kg.[127,128]
Pentasodium Triphosphate	5% in diet for 2 years	Groups of weanling rats (50 males and 50 females/group). Feeding through 3 generations (2 litters produced in each generation)	Normal reproduction and no adverse reproductive effects in offspring.[8]
Pentasodium Triphosphate	Injection (increasing doses of 0.7 to 10 mg, and dose of 30 mg) into air chamber of chick embryo after 24 h and 72 h of incubation	Chick embryos	No effects at any dose after 24 h or 72 h of incubation. [129]
Sodium Hexametaphosphate	5% in diet for 2 years	Groups of weanling rats (50 males and 50 females/group). Feeding through 3 generations (2 litters produced in each generation)	Normal reproduction and no adverse reproductive effects in offspring.[8]
Sodium Hexametaphosphate	Doses (vehicle not stated) up to 370 mg/kg/day on gestation days 6-16	~ 24 albino CD-1 mice	No treatment-related effects (NOEL > 370 mg/kg).[8]
Sodium Hexametaphosphate	Doses (vehicle not stated) up to 138 mg/kg/day on gestation days 6-16	~ 24 Wistar albino rats	No treatment-related effects (NOEL > 138 mg/kg).[8]
Sodium Hexametaphosphate	Injection via the air cell/yolk. Doses up to 10 mg/egg (maximum volume injected = 100 µl). LD_{50} values determined and gross examination for developmental abnormalities performed	100 chick embryos per dose level	LD_{50} = 1.53 mg/egg (air cell injection). Cleft palate and other anomalies at all doses (0.5 to 10 mg/egg). Teratogenic.[130]
Sodium Metaphosphate	Injection (increasing doses of 0.7 to 10 mg, and dose of 30 mg) into air chamber of chick embryo after 24 h and 72 h of incubation	Chick embryos	No effects at any dose after 72 h of incubation. Doses of 10 to 15 mg had lethal effect after 24 h of incubation. Embryos of 2nd and 3rd brooding day had characteristic misshapes of the brain, heart primordium, and somites. Anomalies observed at microscopic examination.[129]
Sodium Phosphate	Injection via the air cell/yolk. Doses up to 10 mg/egg (maximum volume injected = 100 µl). LD_{50} values determined and gross examination for developmental abnormalities performed	100 chick embryos per dose level	LD_{50} = 2 mg/egg (air cell injection); LD_{50} = 0.53 mg/egg (yolk injection). Cleft palate and other anomalies at all doses (0.5 to 10 mg/egg). Teratogenic.[130]

Table 8. Reproductive and Developmental Toxicity Studies

Ingredient	Test Protocol	Animals/Embryos	Results
Sodium Polyphosphate/Sodium Hexametaphosphate	Doses (vehicle not stated) up to 141 mg/kg/day; days of gestation not stated	Rats and mice	No treatment-related effects (NOEL > 141 mg/kg).[114]
Sodium Phosphate	Doses (in water) up to 370 mg/kg/day on gestation days 6-15	19 to 22 CD-1 mice	No treatment-related effects (NOEL > 370 mg/kg).[131]
Sodium Phosphate	Doses (in water) up to 410 mg/kg/day on gestation days 6-15	20 Wistar rats	No treatment-related effects (NOEL > 410 mg/kg).[131]
Sodium Trimetaphosphate	0.1%, 1%, or 10% in diet for 2 years	Weanling rats (number/strain not stated)	At up to 10% in diet, no effect on fertility or litter size through F_2 generation.[61]
Tetrasodium Pyrophosphate	Doses (in corn oil) up to 130 mg/kg/day on gestation days 6-15	18 to 21 CD-1 mice	No treatment-related effects (NOEL > 130 mg/kg).[132]
Tetrasodium Pyrophosphate	Doses (in corn oil) up to 138 mg/kg/day on gestation days 6-15	19 to 21 Wistar rats	No treatment-related effects (NOEL > 138 mg/kg).[132]
Tetrasodium Pyrophosphate	Injection via the air cell/yolk. Doses up to 5 mg/egg (maximum volume injected = 100 µl). LD_{50} values determined and gross examination for developmental abnormalities performed	100 chick embryos per dose level	LD_{50} values: 3.87 mg/egg (air cell injection at 0 h), 0.34 mg/egg (air cell injection at 96 h), and 0.12 mg/egg (yolk sac injection at 0 h). Serious terata reported, including one observation of ectopia cordis. Teratogenic.[130]
Sodium and Potassium Salts			
Tetrasodium Pyrophosphate + Potassium Metaphosphate	0.5% commercial preparation (in Sherman diet) containing 67% Tetrasodium Pyrophosphate and 33% Potassium Metaphosphate (effective concentration [Tetrasodium Pyrophosphate = 0.5% x 67% = 0.34%; effective concentration [Potassium Metaphosphate] = 0.5% x 33% = 0.17%]	Rats (10 males, 10 females). Feeding continued through 2nd and 3rd generations	Growth and fertility were normal. No difference in incidence of abnormalities between treated and control animals.[8,60]
Tetrasodium Pyrophosphate + Potassium Metaphosphate	1% commercial preparation (in Sherman diet) containing 67% Tetrasodium Pyrophosphate and 33% Potassium Metaphosphate (effective concentration [Tetrasodium Pyrophosphate = 1% x 67% = 0.67%; effective concentration [Potassium Metaphosphate] = 1% x 33% = 0.33 %]	Rats (10 males, 10 females). Feeding continued through 2nd and 3rd generations	Growth and fertility were normal. No difference in incidence of abnormalities between treated and control animals.[8,60]

Table 8. Reproductive and Developmental Toxicity Studies

Ingredient	Test Protocol	Animals/Embryos	Results
Tetrasodium Pyrophosphate + Potassium Metaphosphate	5% commercial preparation (in Sherman diet) containing 67% Tetrasodium Pyrophosphate and 33%Potassium Metaphosphate (effective concentration [Tetrasodium Pyrophosphate =5% x 67% = 3.4%; effective concentration [Potassium Metaphosphate] = 5% x 33% = 1.7%]	Rats (10 males, 10 females). Feeding continued through 2nd and 3rd generations	Growth and fertility were normal. No difference in incidence of abnormalities between treated and control animals.[8,60]
	Potassium Salts		
Dipotassium Phosphate	Doses of 1000 mg/kg/day for 42 days (males) and 42 to54 days (females)	Sprague-Dawley rats (males and females)	No reproductive or developmental toxic effects. NOAEL = 1000 mg/kg/day.[18]
Potassium Phosphate	Doses (in water) up to 320 mg/kg/day on gestation days 6-15	20 to 22 CD-1 mice	No treatment-related effects (NOEL > 320 mg/kg).[133]
Potassium Phosphate	Doses (in water) up to 282 mg/kg/day on gestation days 6-15	20 to 25 Wistar rats	No treatment-related effects (NOEL > 282 mg/kg).[133]
Potassium Phosphate	Injection (in water) via the air cell and via the air cell/yolk. Doses up to 10 mg/egg (maximum volume injected = 100 µl). LD_{50} values determined and gross examination for developmental abnormalities performed	100 chicken embryos per dose level	LD_{50} = 1.51 mg/egg. Non-teratogenic.[130]
	Calcium Salts		
Calcium Phosphate	Doses (in water) up to 465 mg/kg/day on gestation days 6-15	19 to 24 CD-1 mice	No treatment-related effects (NOEL > 465 mg/kg).[134]
Calcium Phosphate	Doses (in water) up to 410 mg/kg/day on gestation days 6-15	19 to 22 Wistar rats	No treatment-related effects (NOEL > 410 mg/kg).[134]
Calcium Phosphate	Doses (in water) up to 217 mg/kg/day on gestation days 6-18	9 to 17 Dutch-belted rabbits	No treatment-related effects (NOEL > 217 mg/kg).[134]
Calcium Phosphate	Injection (in 1 N HCl) via the air cell/yolk. Doses up to 2.5 mg/egg (maximum volume injected = 100 µl). LD_{50} values determined and gross examination for developmental abnormalities performed	100 chick embryos per dose level	LD_{50} = 0.37 mg/egg. Non-teratogenic.[130]
Dicalcium Phosphate	Doses of 0, 250, 500, or 1000 mg/kg/day. Males dosed once daily for 2 weeks prior to, during, and post-mating (42 days total). Females dosed once daily for weeks prior to mating, throughout gestation,	Rats (13/sex/dose)	No dose-related effects on mating, gestation, or external malformations. NOAEL of 1,000 mg/kg/day (parents and pups).[88,122]

Table 8. Reproductive and Developmental Toxicity Studies

Ingredient	Test Protocol	Animals/Embryos	Results
	and 4 days after delivery		
Tricalcium Phosphate	Doses of 0, 250, 500, or 1000 mg/kg/day by gavage. Males dosed from 2 weeks before mating to end of mating. Females dosed from 2 weeks before mating to day 4 of lactation (including the mating and gestation periods)	Rats (10/sex/dose)	No treatment-related adverse effects on reproductive parameters and no externally malformed neonates in any dose group. NOAEL for reproductive and developmental toxicity = 1000 mg/kg/day.[56,57]
Tricalcium Phosphate	Injection (in water) via the air cell and via the air cell/yolk. Doses up to 2.5 mg/egg (maximum volume injected = 100 µl)	100 chick embryos per dose level	LD_{50} = 0.85 mg/egg. Non-teratogenic.[130]

Table 9. Genotoxicity Studies

Ingredient/Similar Chemical	Strain/cell type	Assay	Dose/Concentration	Results
		Acids		
Phosphoric Acid	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *E. coli* strain WP2*uvr*A	Ames test	up to 5000 µg/plate	Negative in all strains (with and without metabolic activation).[67,70]
Phosphoric Acid (75%-85% solution)	*Salmonella typhimurium* strains TA97, TA98, TA100, TA102, and TA1535	Ames Test	Concentrations not stated (pHs ranged from 4 to 9)	Negative in all strains (with and without metabolic activation).[70,135]
Phosphoric Acid (75%-85% solution)	*Salmonella typhimurium* strains TA97, TA98, TA100, and TA104	Ames Test	up to 2 µl/plate	Negative in all strains (with and without metabolic activation).[136]
Phosphoric Acid	Chinese hamster lung cells	Chromosome aberrations assay	Up to 450 µg/ml	Negative (with and without metabolic activation).[67,70]
		Ammonium Salts		
Diammonium Phosphate	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* strain WP2*uvr*A	Ames Test	up to 5000 µg/plate	Negative (with and without metabolic activation).[99]
Diammonium Phosphate	Chinese hamster ovary cells	Chromosome aberrations assay	Up to 1230 µg/ml	Negative (with and without metabolic activation).[99]
		Sodium Salts		
Disodium Phosphate	*Salmonella typhimurium* strains TA92, TA94, TA98, TA100, TA1535 and TA1537	Ames Test	up to 100 mg/plate	Negative in all strains (with and without metabolic activation).[137]
Disodium Phosphate	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537	Ames Test	up to 10,000 µg/plate	Negative in all strains (with and without metabolic activation).[138]
Disodium Phosphate	Chinese hamster fibroblasts (CHL cell line)	Chromosome aberrations assay	up to 2 mg/ml	Negative.[137]
Disodium Pyrophosphate	*Salmonella typhimurium* strains TA92, TA94, TA98, TA100, TA1535 and TA1537	Ames Test	up to 10 mg/plate	Negative in all strains (with and without metabolic activation).[137]
Disodium Pyrophosphate	*Salmonella typhimurium* strains TA97, TA98, TA100, TA102, and TA1535	Ames Test	5% (w/v)	Negative in all strains (with and without metabolic activation).[114]
Disodium Pyrophosphate	*Saccharomyces cerevisiae*	*S. cerevisiae* mutation assay	Not stated	Negative (with or without metabolic activation not stated).[114]
Disodium Pyrophosphate	*Salmonella typhimurium* strain TA1530 and *S. cerevisiae* strain D3	Host mediated assay	up to 1400 mg/kg	Negative in both strains.[114]
Disodium Pyrophosphate	Rats	Dominant lethal test	up to 720 mg/kg	Negative.[114]
Disodium Pyrophosphate	Male mice	Mouse translocation	up to 1400 mg/kg	Negative.[114]

Table 9. Genotoxicity Studies

Ingredient/Similar Chemical	Strain/cell type	Assay	Dose/Concentration	Results
		test		
Disodium Pyrophosphate	Chinese hamster fibroblasts (CHL cell line)	Chromosome aberrations assay	up to 0.5 mg/ml	Negative.[137]
Pentasodium Triphosphate	WI-38 human lung cells (without metabolic activation)	*In vitro* cytogenetics assay	up to 10 µg/ml.	Negative.[139]
Pentasodium Triphosphate	Rats (bone marrow cells)	*In vivo* cytogenetics assay	up to 2500 mg/kg	Negative.[139]
Pentasodium Triphosphate	*Salmonella typhimurium* strains his G46 and TA1530, and *S. cerevisiae* strain D3	Host mediated assay (cells inoculated into mice)	up to 2500 mg/kg	Negative.[139]
Pentasodium Triphosphate	Rats	Dominant lethal test	up to 2500 mg/kg	Negative.[139]
Sodium Hexametaphosphate	*Salmonella typhimurium* strains TA1535, TA1537, and TA1538	Ames test	Not stated	Negative in all strains (with and without metabolic activation).[8]
Sodium Polyphosphate/Sodium Hexametaphosphate	*Salmonella typhimurium* strains TA1535, TA1537, and TA1538	Ames Test	up to 0.018 µg/plate	Negative in all strains (with and without metabolic activation).[114]
Sodium Polyphosphate/Sodium Hexametaphosphate	*S. cerevisiae* strain D4	*S. cerevisiae* mutation assay	up to 0.018 µg/plate	Negative (with and without metabolic activation).[114]
Sodium Phosphate	*Salmonella typhimurium* strains TA1535, TA1537, and TA1538	Ames Test	up to 1.25%	Negative in all strains (with and without metabolic activation).[140]
Sodium Phosphate	*S. cerevisiae* strain D4	*S. cerevisiae* mutation assay	up to 5%	Negative (with and without metabolic activation).[140]
Sodium Phosphate	*Escherichia coli* strain WP2uvrA	SOS chromotest (without metabolic activation)	10 to 100,000 nM/ml	Negative.[141,142]
Tetrasodium Pyrophosphate	*Salmonella typhimurium* strains TA1535, TA1537, and TA1538	Ames test	up to 0.1% (w/v)	Negative in all strains (with and without metabolic activation).[139]
Tetrasodium Pyrophosphate	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2uvrA	Ames test	Up to 4820 µg/plate	Negative in all strains (with and without metabolic activation).[143]
Tetrasodium Pyrophosphate	*S. cerevisiae* strain D4	*S. cerevisiae* mutation assay	up to 2.25% (w/v)	Negative (with and without metabolic activation).[139]
Potassium Salts				
Dipotassium Phosphate(liquid)	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538; *S. cerevisiae*	Ames Test	up to 5 µl/plate	Negative in all strains (with and without metabolic activation).[50]

Table 9. Genotoxicity Studies

Ingredient/Similar Chemical	Strain/cell type	Assay	Dose/Concentration	Results
	strain D4			
Dipotassium Phosphate	*Salmonella typhimurium* strains TA97 and TA102	Ames test	Up to ~ 10 mg/plate	Negative (with and without metabolic activation).[18]
Dipotassium Phosphate	Chinese hamster lung cells	Chromosome aberrations assay	Up to 5000 µg/ml	Negative (with and without metabolic activation).[18]
Potassium Phosphate	*Salmonella typhimurium* strains TA1535, TA1537, and TA1538; *S. cerevisiae* strain D4	Ames Test	up to 5% (w/v)	Negative in all strains (with and without metabolic activation).[144]
Tetrapotassium Pyrophosphate	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538; *S. cerevisiae* strain D4	Ames Test	up to 5 µl/plate	Negative in all strains (with and without metabolic activation).[50]
		Calcium Salts		
Calcium Phosphate	*Salmonella typhimurium* strains TA1535, TA1537, and TA1538	Ames Test	up to 0.75%	Negative in all strains (with and without metabolic activation).[145]
Calcium Phosphate	*S. cerevisiae* strain D4	*S. cerevisiae* mutation assay	up to 5% (w/v)	Negative.[145]
Dicalcium Phosphate	*Salmonella typhimurium* strains TA97 and TA102	Ames Test	Not stated	Negative (with or without metabolic activation not stated).[50,146]
Dicalcium Phosphate	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537; *E. coli* strain WP2*uvr*A	Ames test	Up to 2000 µg/plate	Negative (with or without metabolic activation).[88]
Dicalcium Phosphate	Chinese hamster lung fibroblasts (CHL cells)	Chromosome aberrations assay	Up to 500 µg/ml	Not clastogenic (with or without metabolic activation).[88]
Tricalcium Phosphate	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537; *E. coli* strain WP2*uvr*A	Ames test	Up to 1250 µg/plate	Negative (with or without metabolic activation).[13,57]
Tricalcium Phosphate	Chinese hamster lung cells (CHL/IU)	Chromosome aberrations assay	Up to 200 µg/ml	Negative (with or without metabolic activation).[13]

Table 10. Skin Irritation/Sensitization Studies

Ingredient (test concentration, if available)	Test Protocol	Non-humans/Humans (number stated, if available from source)	Results
		Acids	
Animal Studies			
Phosphoric Acid (5% and 30%)	Intracutaneous application (intact skin). 6-h observation period	Juvenile white mice	5% concentration moderately irritating; 30% concentration severely irritating.[70]
Phosphoric Acid (100%)	4-h application (under occlusion) to abraded and intact skin	Rabbits	Corrosive.[50]
Phosphoric Acid (85% solution)	24-h application (under occlusion) to abraded and intact skin	Rabbits	Moderately to severely irritating.[50]
Phosphoric Acid (85% solution)	24-h application	New Zealand white rabbits	Corrosive.[70]
Phosphoric Acid (75%-85%)	24-h application (0.5 ml under semi-occlusive patch)	New Zealand albino rabbits	Corrosive.[147]
Phosphoric Acid (80%)	24-h application (0.5 ml under 1" x 1"occlusive patch) to abraded and intact skin	Rabbits (at least 6)	Highly irritating.[148]
Phosphoric Acid (75%, 80%, and 85%)	4-h application (0.5 ml under 1" x 1"occlusive patch) to abraded and intact skin	Albino rabbits (at least 6)	Non-corrosive (75% and 80%). Corrosive (85%).[149]
Phosphoric Acid (75%)	4-h application (no occlusion) to intact skin	Rabbits	Non-irritating.[50]
Phosphoric Acid (75%)	4-h application (semiocclusion) to intact skin	1 New Zealand white rabbit	Non-irritating.[70]
Phosphoric Acid (70%)	4-h application (under occlusion) to abraded and intact skin	Rabbits	Corrosive.[50]
Phosphoric Acid (52%)	Applied (under occlusion) to abraded and intact skin	Rabbits	Severely irritating and corrosive.[50]
Phosphoric Acid (30%)	Buchner method.[150]	Not stated	Highly irritating.[151]
Phosphoric Acid (19%)	Not stated	2 Rabbits	Non-irritating.[152]
Phosphoric Acid (≥ 17.5% [pH 0.6 to 0.2])	Under occlusion for 4 h	Rabbits	Corrosive (formation of scar tissue).[67]
Phosphoric Acid (2.5%, pH 2.1)	Not stated	3 Rabbits	Severe erythema with mild to moderate swelling (1 rabbit) at 42 h to 72 h after exposure.[67]
Human Studies			
Phosphoric Acid (concentration not stated)	Not stated	Human subjects	Non-sensitizer.[50,68]
		Ammonium Salts	
Animal Studies			
Ammonium Phosphate	4-h application (under occlusion) to abraded and intact skin	Rabbits	Mildly irritating.[50]
Ammonium Phosphate	24-h application (under	Rabbits	Non-irritating.[50]

Table 10. Skin Irritation/Sensitization Studies

Ingredient (test concentration, if available)	Test Protocol	Non-humans/Humans (number stated, if available from source)	Results
	occlusion) to intact skin		
Diammonium Phosphate	4-h application (under occlusion) to abraded and intact skin	Rabbits	Mildly irritating.[50]
Diammonium Phosphate	24-h application (under occlusion) to intact skin	Rabbits	Slightly irritating.[50]
Diammonium Phosphate	24-h application (under occlusion) to intact skin	Rabbits	Non-irritating.[50]
		Sodium Salts	
Animal Studies			
Disodium Phosphate	24-h application (under occlusion) to abraded and intact skin	Rabbits	Moderately irritating (abraded skin) and mildly irritating (intact skin).[50]
Disodium Phosphate	24-h application (under occlusion) to intact skin	Rabbits	Non-irritating.[50]
Disodium Pyrophosphate	24-h application (under occlusion) to intact skin	Rabbits	Mildly irritating.[100]
Disodium Pyrophosphate	4-h application (under occlusion) to abraded and intact skin	Rabbits	Slightly irritating.[50]
Disodium Pyrophosphate	24-h application (under occlusion) to intact skin	Rabbits	Non-irritating.[50]
Pentasodium Triphosphate	4-h application (no occlusion) to intact skin	Rabbits	Slightly irritating.[50]
Pentasodium Triphosphate	24-h application (under occlusion) to abraded and intact skin	Rabbits	Slightly to moderately irritating.[50]
Pentasodium Triphosphate	24-h application (under occlusion) to abraded and intact skin	Rabbits	Moderately irritating.[50]
Pentasodium Triphosphate	24-h application (under occlusion) to abraded and intact skin	Rabbits	Slightly irritating.[50]
Sodium Polyphosphate/Sodium Hexametaphosphate	4-h application (no occlusion) to intact skin	Rabbits	Slightly irritating.[50]
Sodium Polyphosphate/Sodium Hexametaphosphate	24-h application (under occlusion) to intact skin	Rabbits	Non-irritating.[50]
Sodium Phosphate	4-h application (no occlusion) to intact skin	Rabbits	Non-irritating.[50]
Sodium Phosphate	24-h application (under occlusion) to abraded and intact skin	Rabbits	Non-irritating.[50]
Sodium Phosphate	Local lymph node assay. Up to 10% in propylene glycol	Female mice of the CBA/Ca (CBA/CaOlaHsd) strain	Non-sensitizer.[69]
Sodium Trimetaphosphate	24-h application (under occlusion) to intact skin	Rabbits	Non-irritating.[50]
Tetrasodium Pyrophosphate (50% aqueous paste)	24-h application (under occlusion) to intact skin	Rabbits	Irritating.[50]

Table 10. Skin Irritation/Sensitization Studies

Ingredient (test concentration, if available)	Test Protocol	Non-humans/Humans (number stated, if available from source)	Results
Tetrasodium Pyrophosphate (25% aqueous suspension)	24-h application (under occlusion) to abraded and intact skin	Rabbits	Irritating.[50]
Tetrasodium Pyrophosphate	24-h application (under occlusion) to abraded and intact skin	Rabbits	Non-irritating.[50]
Tetrasodium Pyrophosphate	24-h application (under occlusion) to intact skin	Rabbits	Non-irritating[100]
Tetrasodium Pyrophosphate	4-h application (no occlusion) to intact skin	Rabbits	Non-irritating.[50]
Trisodium Phosphate(95% purity)	24-h application (under occlusion) to abraded and intact skin	Rabbits	Minimally irritating (abraded skin) and non-irritating (intact skin).[100]
Trisodium Phosphate(95% purity)	4-h application (under occlusion) to intact skin	Rabbits	Non-irritating.[100]
Trisodium Phosphate(19% solution)	4-h or 24-h application (under occlusion) to abraded and intact skin	Rabbits	Slightly irritating at 4 h and non-irritating at 24 h
Trisodium Phosphate(15% solution)	4-h or 24-h application (under occlusion) to abraded and intact skin	Rabbits	Slightly irritating at 4 h and non-irritating at 24 h
Trisodium Phosphate	24-h application (under occlusion) to abraded and intact skin	Rabbits	Irritating (abraded and intact skin).[50]
Trisodium Phosphate	24-h application (under occlusion) to intact skin	Rabbits	Slightly irritating.[50]
Human Studies			
Pentasodium Triphosphate (50% solution)	Not stated	6 subjects	Negligible irritation potential.[32]
Sodium Metaphosphate (1%)	Application to intact skin	20 subjects (with suspected or verified contact allergy to cosmetic products)	Mild skin irritation.[32]
		Potassium Salts	
Animal Studies			
Dipotassium Phosphate	4-h (under occlusion) or 24-h (no occlusion) application to intact skin	Rabbits	Slightly irritating,[50]
Dipotassium Phosphate	24-h application (under occlusion) to abraded and intact skin	Rabbits	Mildly irritating.[50]
Dipotassium Phosphate	24-h application (under occlusion) to intact skin	Rabbits	Minimally irritating.[50]
Dipotassium Phosphate(liquid)	24-h application (under occlusion) to abraded and intact skin	Rabbits	Slightly irritating.[50]
Pentapotassium Triphosphate	4-h application (no occlusion) to intact skin	Rabbits	Non-irritating.[50]
Potassium Phosphate	4-h application (no occlusion) to intact skin	Rabbits	Non-irritating.[50]

Table 10. Skin Irritation/Sensitization Studies

Ingredient (test concentration, if available)	Test Protocol	Non-humans/Humans (number stated, if available from source)	Results
Potassium Phosphate	4-h application (under occlusion) to abraded and intact skin	Rabbits	Non-irritating.[50]
Potassium Phosphate	24-h application (under occlusion) to intact skin	Rabbits	Slightly irritating.[50]
Tetrapotassium Pyrophosphate	4-h application (no occlusion) to intact skin	Rabbits	Non-irritating.[50]
Tetrapotassium Pyrophosphate	4-h application (under occlusion) to abraded and intact skin	Rabbits	Non-irritating.[50]
Tetrapotassium Pyrophosphate (aqueous solution)	24-h application (under occlusion) to intact skin	Rabbits	Slightly irritating.[50]
Tetrapotassium Pyrophosphate (aqueous solution)	24-h application (under occlusion) to intact skin	Rabbits	Mildly irritating.[50]
Tetrapotassium Pyrophosphate	24-h application (under occlusion) to intact skin	Rabbits	Slightly irritating.[50]
Tetrapotassium Pyrophosphate	24-h application (under occlusion) to intact skin	Rabbits	Non-irritating.[50]
		Calcium Salts	
Animal Studies			
Calcium Dihydrogen Phosphate	24 h application of 0.5 g (wrapped in rubber)	Rabbits (3 males and 3 females)	Non-irritating.[88]
Calcium Phosphate	4-h application (under occlusion) to abraded and intact skin	Rabbits	Mildly irritating.[50]
Calcium Phosphate	4-h application (under occlusion) to abraded and intact skin	Rabbits	Non-irritating.[50]
Calcium Phosphate	24-h application (under occlusion) to intact skin	Rabbits	Non-irritating.[100]
Calcium Pyrophosphate	24-h application (under occlusion) to intact skin	Rabbits	Non-irritating[50]
Dicalcium Phosphate	24-h application (0.5 g, under occlusion) to abraded and intact skin	6 Rabbits	Non-irritating.[69]
Dicalcium Phosphate	24-h application (under occlusion) to intact skin	Rabbits	Non-irritating.[50]
Tricalcium Phosphate	4-h application (no occlusion) to intact skin	Rabbits	Non-irritating.[50]
Tricalcium Phosphate	4-h application (under occlusion) to abraded and intact skin	Rabbits	Slightly irritating.[50]
Tricalcium Phosphate	24-h application (under occlusion) to abraded and intact skin	Rabbits	Non-irritating.[50]

Table 10. Skin Irritation/Sensitization Studies

Ingredient (test concentration, if available)	Test Protocol	Non-humans/Humans (number stated, if available from source)	Results
		Magnesium Salts	
Animal Studies			
Magnesium Phosphate	4-h application (under occlusion) to abraded and intact skin	Rabbits	Non-irritating.[50]
Magnesium Phosphate	4-h application (under occlusion) to intact skin	Rabbits	Non-irritating.[50]
Magnesium Phosphate	24-h application (under occlusion) to abraded and intact skin	Rabbits	Non-irritating.[50]
Trimagnesium Phosphate	24-h application (under occlusion) to abraded and intact skin	Rabbits	Non-irritating.[50]
Trimagnesium Phosphate	24-h application (under occlusion) to intact skin	Rabbits	Non-irritating.[50]

Table 11. Ocular Irritation/Toxicity Studies

Ingredient	Test Protocol	Animals (number stated, if available from source)	Results
		Acids	
Phosphoric Acid (119 mg)	Not stated	Rabbits	Irritating. Risk of serious damage to eyes.[153]
Phosphoric Acid (75%, 80%, and 85% solutions)	Draize Test	3 rabbits	All corrosive.[70,71]
Phosphoric Acid (85%)	Draize Test	Rabbits	Severe irritant.[50]
Phosphoric Acid (70% solution)	Draize Test	Rabbits	Corrosive.[50]
Phosphoric Acid (10% and 17% in water)	OECD Guideline 405. Instilled (100 µl) into lower conjunctival sac	6 New Zealand white albino rabbits	Conjunctivitis observed (both concentrations), but classified as non-irritating.[67,70]
Phosphoric Acid	Irrigation with 0.16 M solution (buffered to pH 3.4)	Rabbits	Slight transient epithelial edema and conjunctival hyperemia.[3]
Metaphosphoric Acid	Injection into corneal stroma or application to cornea after removal of epithelium	Rabbits	Injury detected at < pH 5.5.[3]
		Ammonium Salts	
Ammonium Phosphate	Draize Test	Rabbits	At 24 h, slightly irritating.[50]
Ammonium Phosphate (solution, concentration not stated)	Draize Test	Rabbits	At 24 h, mildly to moderately irritating.[50]
Diammonium Phosphate	Draize Test	Rabbits	At 24 h, slightly irritating to moderately irritating.[99]
		Sodium Salts	
Diodium Phosphate	Draize Test	Rabbits	At 24 h, practically non-irritating (rinsed eyes) and minimally irritating (unrinsed eyes).[50]
Disodium Phosphate	Instilled into eye	Rabbits	Minimal ocular irritation.[32]
Disodium Pyrophosphate	Draize Test	Rabbits	At 24 h, mildly irritating (rinsed eyes) and extremely irritating (unrinsed eyes).[154]
Disodium Pyrophosphate	Instilled into eye (rinsed or unrinsed)	Rabbits	Marked ocular irritation in unrinsed eyes. Minimal-to-mild irritation after ocular rinsing.[32]
Pentasodium Triphosphate	Draize Test	Rabbits	Non-irritating (rinsed eyes) and mildly irritating (unrinsed eyes).[50]
Pentasodium Triphosphate	Draize Test	Rabbits	At 24, irritating.[50]
Sodium Metaphosphate	Not stated	Rabbits	Non-irritating.[32]
Sodium Polyphosphate/Sodium Hexametaphosphate	Draize Test	Rabbits	Non-irritating (rinsed eyes) and minimally irritating (unrinsed eyes)
Sodium Phosphate	Draize Test	Rabbits	At 24 h, practically non-irritating (rinsed eyes) and minimally irritating (unrinsed eyes).[50]
Sodium Phosphate	Instilled into eye	Rabbits	Minimal ocular irritation.[32]
Sodium Trimetaphosphate	Draize Test	Rabbits	At 24 h, slightly irritating.[50]

Table 11. Ocular Irritation/Toxicity Studies

Ingredient	Test Protocol	Animals (number stated, if available from source)	Results
Tetrasodium Pyrophosphate	Draize Test	Rabbits	Minimally irritating (rinsed eyes) and extremely irritating (unrinsed eyes).[154]
Tetrasodium Pyrophosphate (10% solution)	Draize Test	Rabbits	At 24 h, irritating.[50]
Trisodium Phosphate	Draize Test	Rabbits	Moderately irritating (rinsed eyes) and extremely irritating (unrinsed eyes).[154]
Trisodium Phosphate	Draize Test	Rabbits	Slightly irritating (rinsed eyes) and corrosive (unrinsed eyes).[50]
Trisodium Phosphate(15% aqueous solution)	Draize Test	Rabbits	Mildly irritating.[32]
Trisodium Phosphate(10% solution)	Draize Test	Rabbits	At 24 h, irritating.[50]
Potassium Salts			
Dipotassium Phosphate	Draize Test	6 rabbits	Dipotassium Phosphate(0.1 g solid or 0.1 ml liquid) practically non-irritating (rinsed eyes) and mildly irritating (unrinsed eyes).[50]
Pentapotassium Triphosphate	Draize Test	Rabbits	Non-irritating (rinsed eyes) and mildly irritating (unrinsed eyes).[50]
Potassium Phosphate	Draize Test	Rabbits	Non-irritating (rinsed and unrinsed eyes).[50]
Potassium Phosphate	Draize Test	Rabbits	Slightly irritating.[50]
Tetrapotassium Pyrophosphate	Draize test	Rabbits	Mildly irritating (rinsed eyes) and moderately irritating (unrinsed eyes).[50]
Calcium Salts			
Calcium Dihydrogen Phosphate	0.1 g in eye for 24 h	6 New Zealand albino rabbits	Transient, slight erythema. Non-irritating.[88]
Calcium Dihydrogen Phosphate	SkinEthic reconstituted human corneal model. Tissues treated with 30 mg for 10 minutes		Non-irritant.[88]
Calcium Phosphate	Draize Test	Rabbits	Practically non-irritating (rinsed eyes) and moderately irritating (unrinsed eyes).[154]
Calcium Phosphate	Draize Test	Rabbits	Extremely irritating (rinsed and unrinsed eyes).[50]
Calcium Pyrophosphate	Draize Test	Rabbits	At 24 h, slightly irritating.[50]
Dicalcium Phosphate	Draize Test	6 New Zealand rabbits	Slight erythema, fully reversible within 24 h. Non-irritating.[69]
Dicalcium Phosphate	Draize Test	Rabbits	At 24 h, slightly irritating.[50]
Dicalcium Phosphate	Reconstructed human corneal model (human-derived keratinocytes, triplicate tissues) treated with 30 mg for 10 minutes		Relative mean viability of tissues was 102% after exposure. Test material unable to directly reduce MTT. Non-irritant.[69]

Table 11. Ocular Irritation/Toxicity Studies

Ingredient	Test Protocol	Animals (number stated, if available from source)	Results
Dicalcium PhosphateDihydrate	0.1 g in eye for 24 h	3 albino rabbits (1 male and 2 females)	Transient, slight erythema. Low potential for ocular irritation.[88]
Tricalcium Phosphate	Draize Test	Rabbits	Non-irritating (rinsed eyes).[50]
Magnesium Salts			
Magnesium Phosphate	Draize Test	Rabbits	Slightly irritating (unrinsed eyes).[50]
Trimagnesium Phosphate	Draize Test	Rabbits	At 24 h, non-irritating.[50]

References

1. Nikitakis, J. and Lange B. International Cosmetic Ingredient Dictionary and Handbook. 16 *ed*. Washington, DC: Personal Care Products Council, 2016.

2. Andersen, F. A. Final report on the safety assessment of sodium metaphosphate, sodium trimetaphosophate, and sodium hexametaphosphate. *International Journal of Toxicology*. 2001;20(3):75-89.

3. United States Environmental Protection Agency (EPA). Summary review of health effects associated with elemental and inorganic phosphorus compounds: Health issue assessment. Document Number: EPA/600/8-89/072. 1990. pp.1-80. Research Triangle Park, North Carolina: U.S. Environmental Protection Agency.

4. International Plant Nutrition Institute (IPNI). Monoammonium phosphate (MAP). Production. http://www.mosaicco.com/images/NSS__9_Monoammonium_Phos.pdf. Last Updated 2015.

5. Leikan, D. F. and Achorn F. P. Phosphate fertilizers: production, characteristics, and technologies. *Agronomy*. 2005;46:23-50.

6. O'Neil, M. J. The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. 14th Edition *ed*. Whitehouse Station, New Jersey: Merck Research Laboratories, 2006.

7. Momeni, A. and Filiaggi M. J. Synthesis and characterization of different chain length sodium polyphosphates. *Journal of Non-Crystalline Solids*. 2013;382:11-17.

8. International Program on Chemical Safety (IPCS). Toxicological evaluation of certain food additives. World Health Organization (WHO) Food Additives Series, No. 17. 1982. pp.1-22.

9. Cichy, B. Folek S. and Makala H. Manufacture and use of potassium polyphosphates. *Przemysl Chemiczny*. 2008;87(11):1131-1136.

10. Scherzer, S. and Hagin J. Potassium metaphosphate (potassium polyphosphate). *New Fert.Mater.* 1968;182-198.

11. Rogero, S. O. Braga F. J. C. and Higa O. Z. Cytotoxicity test for bioceramics of calcium phosphate. *Materials Science Forum*. 1999;299-300:44-47.

12. Lima, F. R. Mendonca C. X. Alvarez J. C. Ratti G. Lenharo S. L. R. Kahn H. and Garzillo J. M. F. Chemical and physical evaluations of commercial dicalcium phosphates as sources of phosphorus in animal nutrition. *Poultry Science*. 1995;74(10):1659-1670.

13. Organization for Economic Co-operation and Development (OECD). SIDS initial assessment report for SIAM 29 (Hague, netherland, 20-23 October 2009). Tricalcium phosphate (CAS No. 7758-87-4). http://webnet.oecd.org/hpv/ui/Search.aspx. Last Updated 2009.

14. Hashimoto, K. Toda Y. Mitsuyama N. Imamura Y. Udagawa S. and Hashimoto K. Chemical composition of magnesium phosphates prepared by a wet chemical method using the $Mg(NO_3)_2$-KOH-H_3PO_4-H_2O System. *Gypsum & Lime*. 1994;249:137-146.

15. The United States Pharmacopeial Convention. Food Chemicals Codex. 2012. 8th: pp.58-59. Rockville: The United States Pharmacopeial Convention.

16. International Plant Nutrition Institute (IPNI). Monoammonium phosphate (MAP). Production. http://www.mosaicco.com/images/NSS__9_Monoammonium_Phos.pdf. Last Updated 2015.

17. Baig, A. Tao H. Joelle B. Lisa S. Suszcynsky-Meister E. and White D. J. Extrinsic whitening effects of sodium hexametaphosphate - A review including a dentifrice with stabilized stannous fluoride. *Conpendium of Continuing Education in Dentistry*. 2005;29(9 (Supplement 1)):47-53.

18. Organization for Economic Co-operation and Development (OECD). SIDS initial assessment report for SIAM 23 (Jeju, Korea, 17-20 Oct 2006). Dipotassium hydrogenphosphate (CAS No. 7758-11-4). http://webnet.oecd.org/hpv/ui/Search.aspx. Last Updated 2006.

19. Food and Drug Administration (FDA). Information supplied to FDA by industry as part of the VCRP FDA database. 2016. Washington, D.C.: FDA.

20. Personal Care Products Council. Concentration of use by FDA product category: Phosphoric acid and phosphates. Unpublished data submitted by the Personal Care Products Council on 4-9-2015. 2015. pp.1

21. Rothe H, Fautz R, Gerber E, Neumann L, Rettinger K, Schuh W, and Gronewold C. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104. PM:21669261.

22. Bremmer HJ, Prud'homme de Lodder LCH, and van Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. 20200. http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Date Accessed 8-24-2011. Report No. RIVM 320104001/2006. pp. 1-77.

23. Rothe H. Special aspects of cosmetic spray evaluation. Unpublished information presented to the 26 September CIR Expert Panel. Washington D.C. 2011.

24. Johnsen MA. The Influence of Particle Size. *Spray Technology and Marketing*. 2004;14(11):24-27. http://www.spraytechnology.com/index.mv?screen=backissues.

25. Aylott RI, Byrne GA, Middleton, J, and Roberts ME. Normal use levels of respirable cosmetic talc: preliminary study. *Int J Cosmet Sci*. 1979;1(3):177-186. PM:19467066.

26. Russell RS, Merz RD, Sherman WT, and Sivertson JN. The determination of respirable particles in talcum powder. *Food Cosmet Toxicol*. 1979;17(2):117-122. PM:478394.

27. CIR Science and Support Committee of the Personal Care Products Council (CIR SSC). 11-3-2015. Cosmetic Powder Exposure.

28. Food and Drug Administration (FDA). GRAS Substances (SCOGS) Database. http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm. Last Updated 2015.

29. International Plant Nutrition Institute (IPNI). Monoammonium phosphate (MAP). Production. http://www.mosaicco.com/images/NSS__9_Monoammonium_Phos.pdf. Last Updated 2015.

30. Naqvi, T. S. Naqvi M. S. and Singh R. K. Effect of fertilizer diammonium phosphate on liver, kidney and muscle 5'-nucleosidase activity of fresh water teleost fish *Clarius batrachus*. *Biomedical and Environmental Sciences*. 1993;6(4):385-388.

31. Food and Drug Administration. Information for Healthcare Professionals: Oral sodium phosphate (OSP) products for bowel cleansing (marketed as Visicol and OsmoPrep, and oral sodium phosphate products available without a prescription. http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm126084.htm. Last Updated 2008. Date Accessed 5-27-2015.

32. Willhite, C. C. Ball G. L. and Bhat V. S. Emergency do not consume/do not use concentrations for blended phosphates in drinking water. *Human and Experimental Toxicology*. 2013;32(3):241-259.

33. Seok, D. S. Kwon M. Sung H. J. and Park C. B. Acute oral or dermal and repeated dose 90-day oral toxicity of tetrasodium pyrophosphate in Sprague-Dawley (SD) rats. *Environmental Health and Toxicology*. 2011;26:1-9. http://dx.doi.org/10.5620/eht.2011.26.e2011014.

34. Le Bail, A. Hansen T. and Crichton W. A. Tetrapotassium Pyrophosphate g- and d-K_4P_2O7. *Powder Diffraction*. 2013;28(1):2-12.

35. DeLattre, V. F. Factors contributing to adverse soft tissue reactions due to the use of tartar control toothpastes: report of a case and literature review. *Journal of Periodontology*. 1999;70(7):803-807.

36. United States Environmental Protection Agency (EPA). Exemptions from the requirement of a tolerance. Tetrasodium pyrophosphate and tetrapotassium pyrophosphate. 40 CFR 180.1001. 1996.

37. Gupta, R. K. Relyveld E. H. Lindblad E. B. Bizzini B. Ben-Efraim S. and Gupta C. K. Adjuvants - a balance between toxicity and adjuvanticity. *Vaccine*. 1993;11(3):293-306.

38. Food and Drug Administration (FDA). OTC active ingredients. Calcium phosphate, dicalcium phosphate, sodium phosphate, and tricalcium phosphate. http://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm135691.pdf. Last Updated 2010.

39. Lee, J. H. Chang B. Ryu H. and Lee C. A 90-day subchronic toxicity study of beta-calcium pyrophosphate in rat. *Drug and Chemical Toxicology*. 2009;32(3):277

40. Gaffar, A. Blake-Haskins J. and Mellberg J. In vivo studies with a dicalcium phosphate dihydrate/MFP system for caries prevention. *Internatinoal Dental Journal*. 1993;43(1 (Supplement 1)):81-88.

41. Ekholm, M. Hietanen J. Lindqvist C. Rautavuori J. Santavirta S. Salo A. Seppala J. and Suuronen R. Mixture of e-caprolactone-lactide copolymer and tricalcium phosphate: a histological immunohistochemical study of tissue reactions. *Journal of Materials Science: Materials in Medicine*. 1999;10(2):69-74.

42. Food and Drug Administration (FDA). Antacid products for over-the-counter (OTC) human use. Listing of specific active ingredients. 21 CFR 331.11. 2015.

43. United States Food and Drug Administration (FDA). Magnesium phosphate. Proposed affirmation of GRAS status. 21CFR 184.1434. 2014.

44. National Academies of Science Institute of Medicine. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary reference intakes for calcioum, phosphorus, magnesium, vitamin D, and fluoride. http://www.ncbi.nlm.nih.gov. Washington, D.C. Last Updated 1997.

45. Organization for Economic Co-operation and Development (OECD). OECD HPV Chemical Program. SIDS Dossier, approved at SIAM 24 (17-20 April 2007). Monoammonium Phosphate. http://webnet.oecd.org/hpv/ui/Search.aspx. Last Updated 2007.

46. World Health Organization. Phosphoric acid and phosphate salts, JECFA evaluations on phosphoric acid. http://www.inchem.org/documents/jecfa/jecmono/v17je22htm. Geneva, Switzerland. Last Updated 2003. Date Accessed 7-1-2015.

47. Luttrell, W. E. Toxic tips: Phosphoric Acid. *Chemical Health and Safety*. 2004;11(1):35-36.

48. Fairhall, L. T. Toxic contaminants of drinking water. *Public Works*. 1941;72(6):24

49. Gosselin, R. E. et al. The hydrolysis and excretion of polymeric phosphate. *J.Pharmacol.Exp.Ther.* 1952;106:180-192.

50. Weiner, M. L. Salminen W. F. Larson P. R. Barter R. A. Kranetz J. L. and Simon G. S. Toxicological review of inorganic phosphates. *Food and Chemical Toxicology*. 2001;39:759-786.

51. Schreier, K. and Noller H. G. Stoffmechselversuche mit venschiedenen markierten Polyphosphaten. *Naunyn-Schmiedeberg's Arch.Exp.Path.Pharmak.* 1955;227:199-209.

52. World Health Organization (WHO). Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. WHO Food Additives Series No. 5. http://www.inchem.org/documents/jecfa/jecmono/v05je88.htm. Last Updated 1974. Date Accessed 8-7-2016.

53. Ehrenpreis, E. D. Increased serum phosphate levels and calcium fluxes are seen in smaller individuals after a single dose of sodium phosphate colon cleansing solution; a pharmacokinetic analysis. *Ailment Pharmacol.* 2009;29:1202-1211.

54. Ehrenpreis, E. D. Varala K. and Hammon B. Lower weight is a risk factor for calcium phosphate nephropathy with sodium phosphate colonoscopy preparation: a simulation study. *Am.J.Gastroenterol.* 2008;103:S408-S455.

55. Parakkal, D. and Ehrenpreis E. D. Calcium phosphate nephropathy from colonoscopy preparations: Effect of body weight. *American Journal of Gastroenterology*. 2010;105(3):705

56. Organization of Economic Co-operation and Development (OECD). SIDS initial assessment profile. Tricalcium phosphate. http://webnet.oecd.org/hpv/UI/handler.axd?id=023a670f-0ee3-4033-a1e3-388659226b06. Last Updated 2009.

57. Organization for Economic Co-operation and Development (OECD). SIDS Dossier, approved at SIAM 29 (20-23 October 2009). Tricalcium phosphate. http://webnet.oecd.org/hpv/ui/Search.aspx. Last Updated 2009.

58. Hirano, M. Hattori H. Katsuda S. Kaneuji Y. Shinmei Y. Kawamoto Y. and Sugimoto S. Biological tests of calcium phosphate bone paste (CPC95). *Yakuri to Chiryo*. 1998;26(3):275-285.

59. Bibra Toxicology Advice and Consulting. Toxicity profile for phosphoric acid and common inorganic phosphates. http://www.bibra-information.co.uk/downloads/toxicity-profile-for-phosphoric-acid-and-common-inorganic-phosphates-1993/. Surrey, UK. Last Updated 1993.

60. van Each, G. J. Vionke H. H. Wit S. J. and van Genderen H. Die physiologische Winkung von Polyphosphaten. *Arzneimittel-Forsch*. 1957;7:172-175.

61. The Franklin Institue Research Labs. GRAS (Generally Recognized as Safe) food ingredients - Phosphates. PB221224. Report sponsored by the United States Food and Drug Administration (FDA). 2015. Springfield, VA: National Technical Information Service (NTIS).

62. Nair, R. S. Johannsen F. R. Botle H. F. Newton P. E. and Rinehart W. E. Toxicity of calcium sodiuim metaphosphate fiber. II. Chronic inhalation and oncogenicity study. *Fundamental and Applied Toxicology*. 1992;19(1):79-90.

63. Hiasa, Y. Konishi N. Nakaoka S. Nakamura T. Nishii K. and Ohshima M. Promoting effects of potassium dibasic phosphate on early-stage renal carcinogenesis in unilaterally nephrectomized rats treated with N-Ethyl-N-hydroxyethylnitrosamine. *Japanese Journal of Cancer Research*. 1992;83(7):688-694.

64. Nishii, K. A study of modulation by phosphate salts and potassium citrate on rat renal tumorigenesis. *Nara Igaku Zasshi*. 1993;44(3):156-167.

65. Jin, H. Xu C. Lim H. Park S. Shin J. Chung Y. Park S. Chang S. Youn H. Lee K. Lee Y. Ha Y. Chae C. Beck G. R. Jr. and Cho M. High dietary inorganic phosphate increases lung tumorigenesis and alters Akt signaling. *Am.J.Respir.Crit.Care Med.* 2009;179(1):59-68.

66. Higashi, S. Ohsumi T. Ozumi K. Kuroki K. Inokuchi Y. and Masamichi T. Evaluation of cytotoxicity of calcium phosphate cement consisting of a-tricalcium phophate and dicalcium phosphate dihydrate. *Dental Materials Journal*. 1998;17(3):186-194.

67. Organization for Economic Co-operation and Development (OECD). SIDS initial assessment report for SIAM 28 (15-17 April 2009, Paris, France). Phosphoric Acid. http://webnet.oecd.org/hpv/ui/Search.aspx. Last Updated 2009.

68. International Uniform Chemical Information Database (IUCLID). IUCLID Data Sheet. Orthophosphoric Acid. 1995.

69. Organization for Economic Co-operation and Development (OECD). SIDS Dossier, approvd at CoCAM1 (10/10/2011). Calcium hydrogenorthophosphate. 1http://webnet.oecd.org/hpv/ui/Search.aspx. Last Updated 2011.

70. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. OECD Chemical program. SIDS Dossier approved at Siam 28 (15-17 April 2009). Phosphoric acid. http://webnet.oecd.org/hpv/ui/Search.aspx. Last Updated 2009.

71. Randall, D. J. and Robinson E. C. Acute toxicologic evaluation of various concentrations of phosphoric acid. *Journal of the American College of Toxicology*. 1990;B:69-70.

72. Wilkins, E. Dieppe P. Maddison P. and Evison G. Osteoarthritis and articular chondrocalcinosis in the elderly. *Ann.Rheum.Dis.* 1983;42:280-284.

73. Resnick, D. Niwayama G. Goergen T. G. et al. Clinical, radiographic and pathological abnormalities in calcium pyrophosphate deposition disease (CPPD): pseudogout. *Radiology*. 1977;122:1-15.

74. McCarty, D. J. Kohn N. N. and Faires J. S. The significance of calcium phophate crystals in the synovial fluid of arthritic patients: The "pseudogout syndrome.". *Ann.Intern.Med.* 1962;56:711-737.

75. Belsey, J. Epstein O. and Heresbach D. Systematic review: Adverse event reports for oral sodium phosphate and polyethylene glycol. *Alimentary Pharmacology and Therapeutics*. 2009;29(1):15-28.

76. Ehrenpreis, E. D. Parakkal D. Semer R. and Du H. Renal risks of sodium phosphate tablets for colonoscopy preparation: a review of adverse drug reactions reported to the US Food and Drug Administration. *Colorectal Disease*. 2011;13(9):270-275.

77. Mackey, A. C. Green L. St. Amand K. and Avigan M. Sodium phosphate tablets and acute phosphate nephropathy. *American Journal of Gastroenterology*. 2009;104(8):1903-1906.

78. Ladenhauf, H. N. Stundner O. Florian S. and Stefan D. Severe hyperphosphatemia after administration of sodium-phosphate containing laxatives in children: case series and systemic review of literature. *Pediatric Surgery International*. 2012;28(8):805-814.

79. Aasebo, W. Scott H and and Ganss, R. Kidney biopsies taken before and after oral sodium phosphate bowel cleansing. *Nephrol.Dial.Transplant*. 2015;22:920-922.

80. Parent, M. Hua Y. and Siemiatycki J. Occupational risk factors for renal cell carcinoma in Montreal. *Am.J.Ind.Med.* 2000;38(6):609-618.

81. IARC working group. Occupational exposures to mists and vapours from sulfuric acid and other strong inorganic acids. *IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans Vol:54 (1992) pp.41.-130.* (1992 pp 41-130)

82. Block, G. Matanoski G. M. Seltser R. and Mitchell T. Cancer morbidity and mortality in phosphate workers. *Cancer Research*. 1988;48:7298-7303.

83. World Health Organization (WHO). Evaluation of certain food additives and contaminants. Fifty-seventh report of the Joimnt FAO/WHO Expert Committee on Food Additives . WHO Technical Report Series 909. 2002. pp.21Geneva, Switzerland: World Health Organization.

84. Federation of American Societies for Experimental Biology (FASEB). Effects of dietary factors on skeletal integrity in adults: calcium, phosphorus, vitamin D, and protein. 1981. pp.1-75. Bethesda, MD:

85. Environmental Protection Agency (EPA). Integrated Risk information System (IRIS). Reference concentration for chronic inhalation exposure. Phosphoric acid (CASRN 7664-38-2). http://www.epa.gov/iris/subst/0697.htm#studinhal. Last Updated 2015.

86. Aranyi, C. Henry M. C. Vana S. C. Gibbons R. D. and Iversen W. O. Effects of multiple intermittent inhalation exposures to red phosphorus/butyl rubber obscurant smokes in Sprague-Dawley rats. *Inhalation Toxicology*. 1988;(Premiere Issue):65-78.

87. Cold Spring Harbor Protocol. Recipe: Phosphate-buffered saline (PBS). Cold Spring Harb. Protoc. doi:10.1101/pdb.rec8247. 2006.

88. Organization for Economic Co-operation and Development (OECD). SIDS initial assessment report for CoCam 1 (10-12 October 2011, Paris, France). Calcium hydrogenorthophosphate. http://webnet.oecd.org/hpv/ui/Search.aspx. Last Updated 2011.

89. Organization for Economic Co-operation and Development (OECD). OECD HPV Chemical Program, SIDS Dossier, approved at CoCAM1(10/10/2011). Calcium dihydrogen phosphate. http://webnet.oecd.org/hpv/ui/Search.aspx. Last Updated 2011.

90. Organization for Economic Co-operation and Development (OECD). SIDS initial assessment report for SIAM 24, Paris, France, 17-20 April 2007. Phosphates. http://webnet.oecd.org/hpv/ui/Search.aspx. Last Updated 2007.

91. Ellinger, R. H. Phosphates in food processing. Furia, T. In: *Handbook of Food Additives*. Vol. 1. Cleveland, OH: The Chemical Rubber Company; 1972:617-780.

92. Hodge, H. C. Summaries of toxicological data: toxicity studies on phosphates. *Food Cosmet.Toxicol.* 1964;2:147-154.

93. Eichler, O. Handbuch der experimentellen pharmakologie. Springer-Verlag, 1950.

94. IUCLID. IUCLID Data Sheet. Trisodium Phosphate. 1995.

95. American Industrial Hygiene Association (AIHA). Workplace Environmental Exposure level Guide: Trisodium Phosphate. *American Industrial Hygiene Association Journal*. 1982;43:B51-B52.

96. IUCLID. IUCLID Data Sheet. Tetrapotassium Pyrophosphate. 1995.

97. IUCLID. IUCLID Data Set. Dipotassium Phosphate. 1995.

98. IUCLID. IUCLID Data Sheet. Monopotassium Phosphate. 1995.

99. Organization for Economic Co-operation and Development (OECD). OECD HPV Chemical Programme. SIDS Dossier, approved at SIAM 24 (17-20 April 2007). Diammonium Phosphate. http://webnet.oecd.org/hpv/ui/Search.aspx. Last Updated 2007.

100. Weiner, M. Freeman C. McCarty J. D. Kotkoskie L. A. and Fletcher M. J. Dermal toxicity/skin irritation studies on five inorganic phosphates. *Journal of the American College of Toxicology*. 1990;B:47-49.

101. Mcmeniman, N. P. The toxic effect of some phosphate supplements fed to sheep. *Australian Veterinary Journal*. 1973;49(3):150-152.

102. Organization for Economic Co-operation and Development (OECD). SIDS initial assessment profile. Monoammonium phosphate, diammonium phosphate, ammonium polyphosphate, single superphosphate, and triple superphosphate. http://webnet.oecd.org/Hpv/UI/handler.axd?id=a394f471-d429-4a3a-a4cc-556e354363b7. Last Updated 2007.

103. Craig, J. M. Histological and histochemical changes in the kidneys of rats fed a diet with an excess of inorganic phosphate. *Amer.J.Path.* 1957;33:621

104. Hahn, F. and Seifen E. Arzneimittel-Forsch. *Naturwissenschaften*. 1959;9:501-503.

105. Hahn, F. Toxicology of the polyphosphates. *Zeitschrift fur Ernahrungswissenschaft*. 1961;1:55-64.

106. Datta, P. K. Frazer A. C. Sharratt M. and Sammons H. G. Biological effects of food additives: II. Sodium pyrophosphate. *J.Sci.Food Agriculture*. 1962;13:556-566.

107. Hahn, F. Jacobi H. and Seifen E. Chronische futterungsuensuchi mit polyphosphaten. *Naturwissenschaften*. 1956;8:286-289.

108. Hahn, F. Jacobi H. and Seifen E. Do ortho- and polyphosphates show variable compatibilities on chronic feeding? *Naturwissenschaften*. 1958;8:286-289.

109. Dymsza, H. A. Reussner G. and Thiessen R. Effect of normal and high intakes of orthophosphate and metaphosphate in rats. *Journal of Nutrition*. 1959;69:419-428.

110. Nair, K. M. Sesikera B. Ranganathan S. and Sivakumar B. Bioeffect and safety of long-term feeding of common salt fortified with iron and iodine (double fortified salt) in rat. *Nutrition Research*. 1998;18(1):121-129.

111. Ritskes-Hoitinga, J. Lemmens A. G. Danse L. H. J. C. and Beynen A. C. Phosphorus-induced nephrocancinosis and kidney function in female rats. *J.Nutr.* 1989;119:1423-1431.

112. Federation of American Societies for Experimental Biology (FASEB). Evaluation of the health aspects of phosphates as food ingredients. PB262651. 1975. Springfiled, VA: National Technical Information Service (NTIS).

113. Saxton, J. A. Jr. and Ellis G. H. Effects of long-continued ingestion of sodi9um phosphate upon the parathyroids, kidneys and bones of mature rats. *Amer.J.Path.* 1941;17:590

114. Food and Drug Administration (FDA). Evaluation of the health aspects of food ingredeints. National Technical Information Service (NTIS) document number: PB-262-651. 1975.

115. Matsuzaki, H. Uehara M. Suzuki K. Liu Q. L. Sato S. Kanke Y. et al. High phosphorus diet rapidly induces nephrocalcinosis and proximal tubular injury in rats. *J.Nutr.Sci.Vitaminol.* 1997;43:627-641.

116. Organization for Economic Co-operation and Development. SIDS initial assessment profile. Dipotassium hydrogenphosphate. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2012)4/part5&doclanguage=en. Last Updated 2006.

117. Schneider, P. Papritz G. Muller-Peddinghaus R. Bauer M. Lehmann H. Uebergerg H. and Trautwein G. Die Kaliumhydrogenphosphatinduzierte nephropathie des hundes: I. Glomerulare Veranderungen. *Veterinary Pathology*. 1980;17:720-737.

118. Schneider, P. Papritz G. Muller-Peddinghaus R. Bauer M. Lehmann H. Uebergerg H. and Trautwein G. Die Kaliumhydrogenphosphat-induzierte nephropathie des hundes: I. Pathogenese der tubulusatrophie. *Veterinary Pathology*. 1980;17:699-719.

119. Shimoji, N. Matsushima Y. Imaida K. Hasegawa R. Kurokawa Y. and Hayashi Y. Subchronic oral toxicity of potassium pyrophosphate as a preliminary to long-term carcinogenicity studies in F344 rats. *Bulletin of National Institute of Hygienic Sciences*. 1988;106:66-72.

120. Hogan, A. G. Regan W. O. and House W. B. Calcium phosphate deposits in guinea pigs and the phosphorus content of the diet. *J.Nutr.* 1950;41:203-213.

121. Anderson, M. P. et al. Long-term effect of low dietary calcium:phosphate ratio on the skeletons of *Cebus albifrons* monkeys. *J.Nutr.* 1977;107:834-839.

122. Organization for Economic Co-operation and Development (OECD). SIDS initial assessment profile. Calcium hydrogenorthophosphate. http://webnet.oecd.org/Hpv/ui/handler.axd?id=37dea5fd-4afe-4769-a90c-cab3eef9ae4e. Last Updated 2011.

123. Lang, K. Phosphatbedarf und Schaden durch hohe phosphatzufuhr. *Z.Lebensmitt-Untersuch*. 1959;110:450-456.

124. Food and Drug Administration (FDA). Teratologic evaluation of FDA 71-61 (sodium acid pyrophosphate). National Technical Information Service (NTIS) document number: PB-223-831. 1973.

125. Food and Drug Administration (FDA). Teratologic evaluation of FDA 71-46 (sodium tripolyohosphate, anhydrous). NTIS document number: PB-221-808. 1973.

126. Food and Drug Research Laboratories, Inc. Teratologic evaluation of FDA 71-46 (sodium tripolyphosphate, anhydrous). PB221808. 1973. Springfield, VA: National Technical Information Service (NTIS).

127. Food and Drug Administration (FDA). Teratologic evaluation of FDA 71-46 (sodium tripolyphosphate, anhydrous). NTIS dicument number: PB-223-826. 1973.

128. Food and Drug Research Laboratories, Inc. Teratologic evaluation of FDA 71-46 (sodium tripolyphosphate, anhydrous). PB223826. 1973. Springfield, VA: National Technical information Service (NTIS).

129. Karb, B. Effect of polyphosphates on chick embryo development. *Ernaehrungs-Umschau*. 1970;17(7):276-278.

130. Verrett, M. J. Scott W. F. Reynaldo E. F. Alterman E. K. and Thomas C. A. Toxicity and teratogenicty of food additive chemicals in the developing chick embryo. *Toxicology and Applied Pharmacology*. 1980;56(2):265-273.

131. Food and Drug Administration (FDA). Teratologic evaluation of FDA 73-2, monosodium phosphate, anhydrous in mice and rats. NTIS document number: PB-245-527. 1975.

132. Food and Drug Administration (FDA). Teratologic evaluation of FDA 73-1, tetrasodium pyrophosphate, anhydrous in mice and rats. NTIS document number: PB-245-534. 1974.

133. Food and Drug Administration (FDA). Teratologic evaluation of FDA 73-65, monopotassium phosphate in mice and rats. NTIS document number: PB-245-521. 1975.

134. Food and Drug Administration (FDA). Teratologic evaluation of FDA 71-81(monocalcium phosphate monohydrate) in mice, rats, and rabbits. NTIS document number: PB-234-866. 1974.

135. Cipollaro, M. Corsale G. Esposito A. Ragucci E. Staiano N. Giordano G. and Pagano G. Sublethal pH decrease may cause genetic damage to eukaryotic cell: a study on sea urchins and *Salmonella typhimurium*. *Teratogenesis, Carcinogenesis, and Mutagenesis*. 1986;6:275-287.

136. Al-Ani, F. and Al-Lami S. Absence of mutagenic activity of acidity regulators in the Ames *Salmonella*/microsome test. *Mutation Research*. 1988;206:467-470.

137. Ishidate, M. Sofuni T. Yoshikawa K. Hayashi M. Nohmi T. Sawada M. and Matsuoka A. Primary mutagenicity screening of food additives currently used in Japan. *Food and Chemical Toxicology*. 1984;22:623-636.

138. Haworth, S. Lawlor T. Mortelmans K. Speck W. and Zeiger E. *Salmonella* mutagenicity test results for 250 chemicals. *Environmental Mutagenesis*. 1983;1:3-142.

139. Food and Drug Administration (FDA). Mutagenic evaluation of compound FDA 73-1, tetrasodium pyrophosphate. NTIS document number: PB-245-489. 1975.

140. Food and Drug Administration (FDA). Mutagenic evaluation of compound FDA 73-2, monosodium phosphate anhydrous powdered, FCC grade. NTIS document number: PB-245-508. 1975.

141. Quillardet, P. Huisman O. D'Ari R. and Hofnung M. SOS Chromotest, a direct assay of induction of an SOS function in Escherichia coli K-12 to measure genotoxicity. *Proc.Natl.Acad.Sci.* 1982;79:5971-5975.

142. Olivier, P. and Marzin D. Study of the genotoxic potential of 48 inorganic derivatives with the SOS chromotest. *Mutation Research*. 1987;189(3):263-269.

143. Kim, S. Rim K. Kim H. and Yang J. Mutagenicity of octane and tetrasodium pyrophosphate in bacterial reverse mutation (Ames) test. *The Journal of Toxicological Sciences*. 2010;35(4):555-562.

144. Food and Drug Administration (FDA). Mutagenic evaluation of compound FDA 73-65, monopotassium phosphate granular food grade. NTIS document number: PB-245-513. 1975.

145. Food and Drug Administration (FDA). Mutagenic evaluation of compound FDA 71-81, monocalcium phosphate. NTIS document number: PB-245-509. 1975.

146. Fujita, H. and Sasaki M. Mutagenicity of food additives with *Salmonella typhimurium* TA97 and TA102. *Kenku Nenpo-Tokyo-Toritsu Eisei Kenkyusho*. 1987;38:423-430.

147. Environmental Protection Agency (EPA). High Production Volume Information System (HPVIS). Skin irritation study (1977 study). Phosphoric acid (75% -85%). http://www.epa.gov/hpvis/. Last Updated 2015.

148. Environmental Protection Agency (EPA). High Production Volume Information System (HPVIS). Skin irritation study (1980 data). Phosphoric acid (80%). http://www.epa.gov/hpvis/. Last Updated 2015.

149. Environmental Protection Agency (EPA). High Production Volume Information System (HPVIS). Skin irritation study (1977 study). Phosphoric acid (75%, 80%, and 85%). http://www.epa.gov/hpvis/. Last Updated 2015.

150. Buchner, K. Buchner K. E. and Walz D. The topically irritant substance: essentials-bio-tests-predictions. *Agents Actions*. 1981;11(5):515-519.

151. Environmental Protection Agency (EPA). High Production Volume Information System (HPVIS). Skin irritation study (no date). Phosphoric acid (30%). http://www.epa.gov/hpvis/. Last Updated 2015.

152. Environmental Protection Agency (EPA). High Production Volume Information System (HPVIS). Skin irritation study (1980 study). Phosphoric acid (19%). http://www.epa.gov/hpvis/. Last Updated 2015.

153. Environmental Protection Agency (EPA). High Production Volume information System (HPVIS). Ocular irritation study (1970 study). Phosphoric acid (119 mg). http://www.epa.gov/hpvis/. Last Updated 2015.

154. Weiner, M. Freeman C. McCarty J. D. Kotkoskie L. A. and Fletcher M. J. Eye irritation studies on five inorganic phosphates. *Journal of the American College of Toxicology*. 1990;B:47-49.

2017 FDA VCRP Data

Ammonia

03G - Other Eye Makeup Preparations	1
05A - Hair Conditioner	2
05D - Permanent Waves	5
05F - Shampoos (non-coloring)	1
05I - Other Hair Preparations	2
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	465
06B - Hair Tints	115
06G - Hair Bleaches	2
08G - Other Manicuring Preparations	1
12A - Cleansing	2
12C - Face and Neck (exc shave)	3
Totals	**599**

Ammonium Hydroxide

03B - Eyeliner	22
03D - Eye Lotion	1
03F - Mascara	16
03G - Other Eye Makeup Preparations	3
05A - Hair Conditioner	7
05C - Hair Straighteners	13
05D - Permanent Waves	34
05F - Shampoos (non-coloring)	11
05G - Tonics, Dressings, and Other Hair Grooming Aids	2
05I - Other Hair Preparations	5
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	1075
06B - Hair Tints	1
06F - Hair Lighteners with Color	3
06G - Hair Bleaches	9
06H - Other Hair Coloring Preparation	16
07C - Foundations	1
07I - Other Makeup Preparations	1
08E - Nail Polish and Enamel	2
08G - Other Manicuring Preparations	1
10E - Other Personal Cleanliness Products	1
12A - Cleansing	19
12B - Depilatories	1
12C - Face and Neck (exc shave)	46
12D - Body and Hand (exc shave)	13
12F - Moisturizing	11
12G - Night	16
12H - Paste Masks (mud packs)	1
12I - Skin Fresheners	4
12J - Other Skin Care Preps	18

13A - Suntan Gels, Creams, and Liquids	1
Totals	**1,354**

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: February 2, 2017

SUBJECT: Concentration of Use by FDA Product Category: Ammonia and Ammonium Hydroxide

Concentration of Use by FDA Product Category – Ammonia and Ammonium Hydroxide

Ingredient	Product Category	Maximum Concentration of Use
Ammonia	Hair straighteners (5C)	1.4%
Ammonia	Permanent waves (5D)	0.73-1.4%
Ammonia	Tonics, dressings and other hair grooming aids Not spray (5G)	0.73% 0.00006%
Ammonia	Hair dyes and colors (6A)	2.8-4.6%
Ammonia	Nail creams and lotions (8C)	0.00075%
Ammonia	Other manicuring preparations (8G)	0.00008%
Ammonia	Skin cleansing (cold creams, cleansing lotions, liquids and pads) (12A)	0.086%
Ammonia	Depilatories (12B)	0.00015%
Ammonia	Face and neck products (12C) Not spray	 0.14%
Ammonia	Body and hand products (12D) Not spray	 0.00002%
Ammonia	Other skin care preparations (12J)	0.00008-0.00068%
Ammonium Hydroxide	Eyebrow pencils (3A)	0.58%
Ammonium Hydroxide	Eyeliners (3B)	0.028-0.3%
Ammonium Hydroxide	Eye shadows (3C)	0.022%
Ammonium Hydroxide	Eye lotions (3D)	0.57%
Ammonium Hydroxide	Mascara (3F)	0.083-0.45%
Ammonium Hydroxide	Hair conditioners (5A)	0.00028-0.34%
Ammonium Hydroxide	Hair straighteners (5C)	3.6%
Ammonium Hydroxide	Permanent waves (5D)	1-1.4%
Ammonium Hydroxide	Shampoos (noncoloring) (5F)	0.98-2.7%
Ammonium Hydroxide	Tonics, dressings and other hair grooming aids (5G)	1.3%
Ammonium Hydroxide	Hair dyes and colors (6A)	2.5-12.5%
Ammonium Hydroxide	Other hair coloring preparations (6H)	0.0083%
Ammonium Hydroxide	Foundations (7C)	0.6%
Ammonium Hydroxide	Other manicuring preparations (8G)	0.003-1.2%
Ammonium Hydroxide	Bath soaps and detergents (10A)	0.0012%
Ammonium Hydroxide	Aftershave lotions (11A)	0.57%
Ammonium Hydroxide	Skin cleansing (cold creams, cleansing lotions, liquids and pads) (12A)	0.012-1.2%
Ammonium Hydroxide	Face and neck products Not spray (12C)	 0.45-1.5%
Ammonium Hydroxide	Body and hand products (12D) Not spray	 0.96%
Ammonium Hydroxide	Moisturizing products (12F) Not spray	 0.6%
Ammonium Hydroxide	Night products (12G) Not spray	 0.53%
Ammonium Hydroxide	Paste masks and mud packs (12H)	1.7%

Ammonium Hydroxide	Skin fresheners (12I)	0.29%
Ammonium Hydroxide	Other skin care preparations (12J)	0.14-1.5%

Information collected in 2016-2017

Table prepared: February 1, 2017

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D., Interim Director
COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: August 4, 2017

SUBJECT: Scientific Literature Review Safety Assessment of Ammonia and Ammonium Hydroxide as Used in Cosmetics (CIR report released July 7, 2017)

Key Issues

Although Table 3 indicates that most of the reported uses are in hair coloring products, the text does not clearly stated that the main use of Ammonia and Ammonium Hydroxide in cosmetics is in hair coloring products. For Ammonia 582/599 (97.2%) reported uses were in hair coloring products. For Ammonium Hydroxide, 1104/1354 (81.6%) reported uses were in hair coloring products. As stated in the cosmetic use section, the highest use concentrations for both ingredients is in hair dyes and colors. The predominance of use in hair coloring products should be made clear in the Cosmetic Use section and the Summary.

The Introduction should mention that Diammonium Phosphate has been reviewed by CIR with a conclusion of safe when formulated to be non-irritating as part of the 2016 final report on phosphoric acid and its salts.

If the CIR Expert Panel considers data on Ammonium Chloride and Ammonium Sulfate to be appropriate for this report, perhaps theses ingredients should be added to the report as they are INCI names.

Definition and General Characterization, Summary - CIR does not review ingredients by function. The report should clearly state that "external analgesic" is not an appropriate function in a cosmetic product, but it is not necessary to state that "the only function of Ammonia under review herein is pH adjustor."

Although the Journal may want all references placed at the end of a paragraph, it would be helpful, at least in the early drafts of the report, to place the reference with the information that came from the reference. This would make it easier to find a reference if more details about a study were needed. For example, reference 67 is included at the end of the short-term, inhalation section. The title of reference 67 is: "The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus".

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

There is no mention of chickens in either the short-term inhalation section or Table 6. Was any information from reference 67 actually included in the CIR report?

Chronic, Carcinogenicity, Table 8 - The description of the rat dietary study of Ammonium Sulfate is misleading as it suggests that there was a carcinogenic effect. The dossier submitted to ECHA cites this study to a published reference:

Ota Y, Hasumura M, Okamura M et al. 2006. Chronic toxicity and carcinogenicity of dietary administered ammonium sulfate in F344 rats. *Fd Chem Toxicol* 44:17-27. (available in Carol's office).

The published study makes it clear that two studies were completed, a 52-week toxicity study in which animals were fed diets containing 0, 0.1, 0.6 or 3% Ammonium Sulfate and a 104-week carcinogenicity study in rats fed diets containing 0, 1.5 or 3.0% Ammonium Sulfate. The conclusion of this study (which should be included in the CIR report) states: "In conclusion, the no observed adverse effect level (NOAEL) for ammonium sulfate was estimated from the chronic toxicity of F344 rats to be 0.6% in both sexes, which is equivalent to 256 and 284 mg/kg/day in males and females, respectively, and no evidence of long-term carcinogenic activity was found in the carcinogenicity study." The primary reference should be used as a reference to summarize these studies in the CIR report.

Additional Considerations

Method of Manufacture - It is not clear what is meant by "producer gas". It would be clearer if this was changed to "a gas containing a source of nitrogen".

Cosmetic Use - Although it is correct that Ammonium Hydroxide is not in Annex II (list of substances that cosmetics must not contain), it should be noted that COSING includes Ammonium Hydroxide with Ammonia as part of Annex III entry number 4 (it has the same limitations as Ammonia).

Toxicokinetics, Human, Oral - As the paragraph in this section describes amino acid metabolism which results in the production of Ammonia, the route of exposure is not necessary as a subheading. Stating that "Ammonia is highly toxic" is not appropriate for this section.

Acute, Oral - Were the range of effects observed in these studies dependent on concentration and/or pH?

Acute, Inhalation - What was the range of LC_{50}s reported? The lowest LC_{50} value rather than the highest value should be stated in the text.

Short-Term, Inhalation - Please state the hours/day, days/week for each study.

Subchronic, Inhalation, Summary - Please include the hours/day, days/week the rats were exposed in the 3 month study.

Chronic, Oral - Please clarify what is meant by "as (w/w/t as Ammonium Hydroxide)"? Note w/w/t also appears in other sections of the report, if it is correct, the meaning of this abbreviation should be explained the first time it appears.

Chronic, Inhalation, Human, Risk Assessment - This section states: "To determine the exposure levels, exposed and control workers were sampled over one work shift; the average sample collection period was 8.4 hours." It is not clear what was sampled, breathing zone

air of the workers using personal air monitors?

Developmental and Reproductive Toxicity - This section should also note whether or not adverse effects were observed in the dams.

Genotoxicity, Summary - What were the exposure concentrations in the *in vivo* genotoxicity study in workers?

Carcinogenicity - The tumor promotion studies are not mentioned in the first paragraph. Therefore, references 88 and 89 should not be associated with this paragraph.

Neurotoxicity - In the first paragraph, it should be made clear that hyperammonemia is a metabolic disturbance. What blood levels of Ammonia are considered to be neurotoxic?

Permeation of the Blood Brain Barrier - This information should be in the Toxicokinetics section.

Immunological Effects, Neurological Effects, Summary - Please state the hours/day, days/week of the exposures.

Ocular Irritation - The only reference at the end of the first paragraph is 114 which is a book on alternative methods. The paragraph includes descriptions of rabbit studies. What are the correct references for the rabbit studies?

Case Reports - The information about Ammonia and Alzheimer's disease patients is not a case report.

Other Clinical Reports - It is not clear why the human exposure studies are not presented in the appropriate duration sections under a Human subheading.

Summary - It would be helpful if the Summary would state normal versus excessive blood levels of Ammonia.

Table 5 - There are a number of studies on the second last page of Table 5 for which the duration of the study is not stated.

Table 6 - Two values of product of concentration and exposure time are given in the Results column (390,000 ppm-h, 1,014,000 ppm-h) (references 46, 63), while only one (1,014,000 ppm-h) is stated in the Ingredient column.

How many animals of each species were used in the last study included in Table 6?

Table 8 - What was studied in reference 85 to result in a conclusion of "No evidence of carcinogenic effect" as 4 weeks is not a long enough exposure period to result in a carcinogenic effect.

What was the duration and dose of the study in which mice were dosed with Ammonium and pyrocarbonate? If this is the same study as the first entry of Table 8, it should be presented with the first entry.