International Journal of Toxicology
2022, Vol. 41(Supplement 1) 80S–105S

DOI: 10.1177/10915818221087202
journals.sagepub.com/home/ijt
SAGE

Safety Assessment of Carbonate Salts as Used in Cosmetics

Wilbur Johnson*, Wilma F. Bergfeld†, Donald V. Belsito†, Ronald A. Hill‡, Curtis D. Klaassen†, Daniel C. Liebler†, James G. Marks‡, Ronald C. Shank†, Thomas J. Slaga†, Paul W. Snyder†, Lillian J. Gill§, and Bart Heldreth¶

Abstract

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 6 carbonate salts which function as absorbents, bulking agents, opacifying agents, pH adjusters, buffering agents, abrasives, and oral care agents in cosmetic products. The Panel reviewed relevant data relating to the safety of these ingredients, and concluded that these carbonate 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 6 carbonate salts as used in cosmetics is reviewed in this safety assessment:

Magnesium Carbonate
Ammonium Bicarbonate
Ammonium Carbonate
Calcium Carbonate
Potassium Bicarbonate
Potassium Carbonate

According to the *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), the functions of these ingredients in cosmetic products include: absorbents, bulking agents, opacifying agents, pH adjusters, buffering agents, abrasives, and oral care agents (Table 1).[1] Ingredient definitions are also included in Table 1.

The Expert Panel for Cosmetic Ingredient Safety (Panel) has evaluated the safety of Sodium Sesquicarbonate, Sodium Bicarbonate, and Sodium Carbonate in cosmetic products, and concluded that these ingredients are "safe as presently used in cosmetics."[2] A final report with this conclusion was published in 1987. Subsequently, during a Panel re-review of the safety of these ingredients in 2004, the conclusion originally determined by the Panel was reaffirmed.[3]

Data from chemical registration dossiers submitted to the European Chemicals Agency (ECHA) that relate to some of the ingredients that are being reviewed were found on the ECHA website. A chemical registration dossier may contain data on the cosmetic ingredient that is being reviewed or pertinent data on a surrogate chemical. ECHA data, whether on the cosmetic ingredient or on a surrogate chemical, are included and referenced in the report text.

Chemistry

Definition and General Characterization

The carbonate salts are alkaline salts that may be formed by treating carbonic acid with an appropriate base (e.g., adding carbonic acid to sodium hydroxide will produce sodium carbonate (and water); Figure 1).

However, most of these salts are also naturally occurring as minerals. All of the ingredients in this report are related as either alkaline earth metal (column I or II) or ammonium salts of carbonic acid. This group comprises carbonate salts with differences in properties that can be attributed to differences in the cation component. Assessing the safety of all of these ingredients in a single report facilitates a coherent analysis, taking into account comparabilities and differences in properties among these ingredients. This enables a more informative and efficient safety assessment of the ingredients than would be likely in separate reports that each assesses a single ingredient. The definitions of the carbonate salts that are included in this safety assessment are presented in Table 1. These salts are classified as generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) for use in food. Daily consumption of these GRAS foods would

¶Cosmetic Ingredient Review Executive Director

*Former Cosmetic Ingredient Review Senior Scientific Analyst/Writer
†Expert Panel for Cosmetic Ingredient Safety Member
‡Expert Panel for Cosmetic Ingredient Safety Former Member
§Former Director Cosmetic Ingredient Review

Corresponding Author:
Bart Heldreth, Executive Director, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.
Email: cirinfo@cir-safety.org

result in much larger systemic exposures than what is expected from use in cosmetic products, even if there was 100% dermal absorption of the cosmetic product. Thus, the primary focus of the safety assessment is the review of the safety of topical exposure to these ingredients.

Physical and Chemical Properties

These ingredients are typically colorless or white solids with low formula weights (Table 2). While the carbonate salts may be fairly alkaline in concentrated solution, an acceptable pH can be easily obtained in formulation.

Method of Manufacture

Ammonium Carbonate. Ammonium Carbonate may be prepared from gaseous ammonia, carbon dioxide, and steam.[4]

Calcium Carbonate

Calcium Carbonate, as used for industrial purposes, is extracted by mining or quarrying.[5] Pure Calcium Carbonate can be produced from marble, or it can be prepared by passing carbon dioxide into a solution of calcium hydroxide. In the latter case, Calcium Carbonate is derived from the mixture, forming a grade of product called "precipitated calcium carbonate" or PCC. PCC has a very fine and controlled particle size, diameter of 2 μm, and is particularly useful in the production of paper. The other primary type of industrial product is "ground calcium carbonate" or GCC. The production of GCC involves crushing and processing limestone to create a powdery form graded by size and other properties for many different industrial and pharmaceutical applications.

Composition/Impurities

Magnesium Carbonate. The following specifications for Magnesium Carbonate are referenced in the "Evaluation of the Health Aspects of Magnesium Salts as Food Ingredients" by the Select Committee on GRAS Substances: not less than 40% and not greater than 43.5% magnesium oxide, not greater than 3 ppm arsenic, not greater than 30 ppm heavy metals, not greater than 10 ppm lead, and not greater than 0.6% calcium oxide.[6]

The *Food Chemicals Codex* specification for Magnesium Carbonate impurities states that this chemical should contain no more than 2 mg/kg lead and no more than 0.6% calcium oxide.[7]

Ammonium Bicarbonate

According to the *Food Chemicals Codex*, Ammonium Bicarbonate contains not less than 99% and not more than 100.5% NH_4HCO_3.[7] The following specifications for impurities in Ammonium Bicarbonate are stated as follows: chloride (≤0.003%), lead (≤3 mg/kg), and sulfate (≤0.007%).

Ammonium Carbonate

According to the *Food Chemicals Codex*, Ammonium Carbonate consists of ammonium bicarbonate (NH_4HCO_3) and ammonium carbamate (NH_2COONH_2), and should contain not less than 30% NH_3 and not more than 43% NH_3. The specifications for Ammonium Carbonate impurities are as follows: chloride (≤0.003%), lead (≤3 mg/kg), and sulfate (≤0.005%).[7]

Calcium Carbonate

According to the *Food Chemicals Codex*, Calcium Carbonate contains not less than 98% and not more than 100.5% $CaCO_3$ on a dried basis.[7] The following specifications for impurities in Calcium Carbonate are stated as follows: acid - insoluble substances (≤ 0.2%), arsenic (≤3 mg/kg), fluoride (≤0.005%), lead (≤3 mg/kg), and magnesium and alkali salts (≤1%).

The European Commission's purity criteria for Calcium Carbonate as a color for use in foodstuffs are as follows: loss on drying (≤2%), acid-insoluble substances (≤0.2%), magnesium and alkali salts (≤1.5%), fluoride (≤50 mg/kg), antimony (≤100 mg/kg, singly or in combination), copper (≤100 mg/kg, singly or in combination), chromium (≤100 mg/kg, singly or in combination), zinc (≤100 mg/kg, singly or in combination), barium (≤100 mg/kg, singly or in combination), arsenic (≤3 mg/kg), lead (≤10 mg/kg), and cadmium (≤1 mg/kg).[8]

Potassium Bicarbonate and Potassium Carbonate

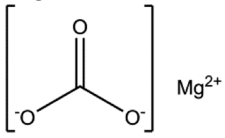
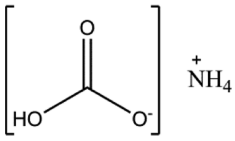
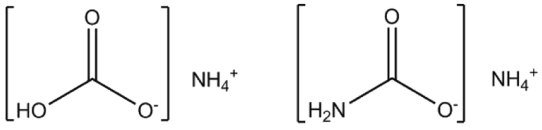
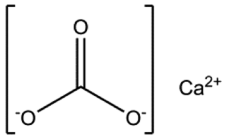
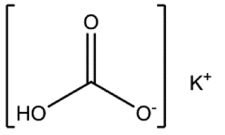
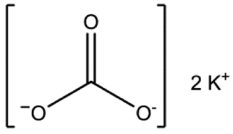
According to the *Food Chemicals Codex*, Potassium Bicarbonate is not less than 99% and not more than 101.5% $KHCO_3$, calculated on the dried basis, and Potassium Carbonate is not less than 99% and not more than 100.5% K_2CO_3 on the dried basis.[7] The specification for impurities in Potassium Bicarbonate and Potassium Carbonate states that each chemical should contain no more than 2 mg/kg lead.

USE

Cosmetic

The safety of the carbonate salts included in this assessment is evaluated based on data received from the US FDA and the cosmetics 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

Table 1. Definitions, Structures, and functions of the ingredients in this safety assessment (INCI Dictionary[1, CIR Staff]).

Ingredient/CAS No.	Definition & Structure	Function
Magnesium Carbonate 546-93-0 7757-69-9	Magnesium Carbonate is a basic dehydrated Magnesium Carbonate or a normal hydrated Magnesium Carbonate. 	Absorbents; Bulking Agents; Opacifying Agents; pH Adjusters
Ammonium Bicarbonate 1066-33-7	Ammonium Bicarbonate is an inorganic salt that conforms to the formula: 	pH Adjusters
Ammonium Carbonate 10361-29-2 (unspecified) 506-87-6 (diammonium) 8000-73-5 (mixture)	Ammonium Carbonate is a mixture of Ammonium Bicarbonate and ammonium carbamate. 	Buffering Agents; pH Adjusters
Calcium Carbonate 471-34-1	Calcium Carbonate is the inorganic salt that conforms to the formula: 	Abrasives; Buffering Agents; Bulking Agents; Opacifying Agents; Oral Care Agents
Potassium Bicarbonate 298-14-6	Potassium Bicarbonate is the inorganic salt that conforms to the formula: 	Buffering Agents; pH Adjusters
Potassium Carbonate 584-08-7	Potassium Carbonate is the inorganic salt that conforms to the formula: 	pH Adjusters

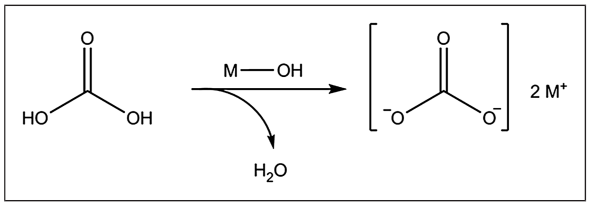


Figure 1. Carbonic Acid and Salts Thereof, wherein "M+" is a cation (e.g., sodium, ammonium, etc.).

maximum reported use concentrations by product category. Collectively, the use frequency and use concentration data (Table 3) indicate that 5 of the 6 ingredients in this safety assessment are currently being used in cosmetic products; Potassium Bicarbonate is not reported as being used.

According to 2016 VCRP data, the greatest reported use frequency is for Magnesium Carbonate (317 product formulations, mostly leave-on products), followed by Calcium Carbonate (174 product formulations, mostly leave-on

Table 2. Properties of Carbonate Salts.[4]

Property	Value	Background Information
Magnesium Carbonate		
Formula Weight (g/mol)	84.31[46]	
Density (g/ml)	3.0[47]	
Melting Point (°C)	900[47]	
Ammonium Bicarbonate		
Form/Odor	Shiny, hard, colorless or white prisms or crystalline mass. Faint odor of ammonia.	Comparatively stable at room temperature. Volatile with decomposition at ~ 60°. Decomposes in hot water.
Formula Weight (g/mol)	79.06	
Solubility	Soluble in water: 14% (10°C); 17.4% (20°C); 21.3% (30°C). Insoluble in alcohol and acetone.	
Ammonium Carbonate		
Form	Flat, columnar, prismatic crystals or elongated flakes.	Commercial preparations are usually a mixture with ammonium carbamate and Ammonium Bicarbonate.
Formula Weight (g/mol)	79.06-78.07	
Melting Point (°C)	43	
Calcium Carbonate		
Form	Odorless, tasteless powder or crystals	Two crystal forms are of commercial importance: Aragonite (orthorhombic; melting point: 825°C (decomposes); density: 2.83; formed at temperatures above 30°; Calcite (hexagonal-rhombohedral; melting point 1339°C; density: 25.2; formed at temperatures below 30°.
Formula Weight (g/mol)	100.09	
Solubility	Soluble in 1N acetic acid, 3N hydrochloric acid, 2N nitric acid. Practically insoluble in water. Insoluble in ethanol.	
Potassium Bicarbonate		
Form	Colorless, transparent crystals, white granules or powder.	Contains not less than 99% $KHCO_3$.
Formula Weight (g/mol)	100.11	
Water Solubility (g/l)		
@ 20°C & pH 7	357	
@ 50°C & pH 7	500	
Solubility	Almost insoluble in ethanol.	
Potassium Carbonate		
Form	Hygroscopic, odorless granules or granular powder.	
Formula Weight (g/mol)	138.20	
Potassium Carbonate		
Solubility	Soluble in 1 part cold water and in 0.7 part boiling water. Practically insoluble in alcohol.	
Density (g/ml)	2.29	
Melting Point (°C)	891	

Table 3. Frequency and Concentration of Use According to Duration and Type of Exposure.[9,10]

	Magnesium Carbonate		Ammonium Bicarbonate		Ammonium Carbonate	
	# Of Uses	Conc. (%)	# Of Uses	Conc. (%)	# Of Uses	Conc. (%)
Totals/Conc. Range	317	0.1-14.4	70	3-93.4	27	0.6-6.5
Duration of Use						
Leave-On	225	0.1-7	2	NR	3	0.6-3
Rinse off	90	0.12-14.4	68	3-93.4	24	2-6.5
Diluted for (bath) Use	2	NR	NR	NR	NR	NR
Exposure Type						
Eye Area	70	0.2-2	NR	NR	NR	NR
Incidental Ingestion	2	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	9*	0.18	1***	NR	3*	NR
Incidental Inhalation- Powders	109	0.5-4	1***	NR	NR	NR
Dermal Contact	283	0.1-7	1	NR	1	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	3	NR	9	3-6.1	2	0.6-3
Hair-Coloring	27	0.12-14.4	60	1.5-93.4	24	2-6.5
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	5	NR	NR	NR	NR	NR
Baby Products	3	NR	NR	NR	NR	NR

	Calcium Carbonate		Potassium Carbonate	
	# of uses	Conc. (%)	# of uses	Conc. (%)
Totals/Conc. Range	174	0.0036-35	4	0.0019-2.5
Duration of Use				
Leave-On	113	0.001-35	1	0.002-0.0068
Rinse off	59	0.0036-25	3	0.0019-2.5
Diluted for (bath) Use	2	NR	NR	NR
Exposure Type				
Eye Area	16	2-35	NR	0.002-0.005
Incidental Ingestion	41	0.045-10	NR	NR
Incidental Inhalation- Sprays	17*	NR	1*	NR
Incidental Inhalation- Powders	42	0.047-15; 25**	NR	0.006-0.0068**
Dermal Contact	129	0.001-35	4	0.0019-0.0068
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	1	0.01-6	NR	NR
Hair-Coloring	NR	<0.01-8	NR	2-2.5
Nail	1	10-15	NR	NR
Mucous Membrane	54	0.0036-10	NR	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.

products) (Table 3).[9] The results of a concentration of use survey conducted in 2015 indicate that Ammonium Bicarbonate has the highest maximum concentration of use; it is used at concentrations up to 93.4% in rinse-off products (hair bleaches) The maximum concentration of use in leave-on products is being reported for Calcium Carbonate (concentrations up to 35% in eyebrow pencils) (Table 3).[10]

Cosmetic products containing carbonate salts may be applied to the skin and hair or, incidentally, may come in contact with the eyes (e.g., Calcium Carbonate at maximum use concentrations up to 35% in eye area cosmetics) and mucous membranes (e.g., Calcium Carbonate at maximum use concentrations up to 10% in dentifrices). Additionally, some of these ingredients are being used in products that may result in incidental ingestion. For example, Calcium Carbonate is being used in dentifrices at maximum use concentrations up to 10%, and in lipstick at maximum use concentrations up to 8%. 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.

Magnesium Carbonate is used in aerosol color hair sprays at maximum use concentrations up to 0.18%, and in face powders at maximum use concentrations up to 4%. Calcium Carbonate is used in powders (dusting and talcum, excluding aftershave talc) at maximum use concentrations up to 5%, and in face powders at maximum use concentrations up to 15%. 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.[11-14] 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.[11,12] 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.[15-17]

Calcium Carbonate and Magnesium Carbonate appear on the list of colorants allowed in cosmetic products that are marketed within the European Union.[18] The purity criteria that have been established for Calcium Carbonate are presented in the Composition/Impurities section of this safety assessment.[8] These criteria must be met for Calcium Carbonate when this ingredient is used as a colorant in cosmetic products.

Noncosmetic

The following carbonate salts are direct food additives that are classified as GRAS in the United States: Magnesium Carbonate, Ammonium Bicarbonate, Ammonium Carbonate, Calcium Carbonate, Potassium Bicarbonate, and Potassium Carbonate.[19]

The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives has determined that Magnesium Carbonate, Ammonium Carbonate, Calcium Carbonate, and Potassium Carbonate are not limited in terms of overall daily intake (mg/kg body weight).[20] The Committee noted that these bases are required for pH adjustment in food technology, and that the amounts and concentrations used are not likely to have any toxicological significance. Furthermore, the Committee placed no restriction on the food-additive use of these bases, provided that the contribution made to the dietary load of potassium, calcium, and magnesium is assessed and considered to be acceptable.

A grade of Calcium Carbonate that is referred to as "precipitated calcium carbonate" or PCC is particularly useful in the production of paper.[5] Combinations of viscous xylocaine, aluminum hydroxide-Magnesium Carbonate, and diphenhydramine hydrochloride have been used to treat mucosal toxicity resulting from chemotherapy and radiotherapy in the treatment of esophageal cancer.[21]

Carbonate salts are also used as inactive ingredients in FDA-approved drug products, and these uses are summarized below:

Magnesium Carbonate

Magnesium Carbonate is used as an inactive ingredient (maximum potency of 10 mg to 250 mg) in oral drug products that have been approved by FDA.[22]

Calcium Carbonate

Calcium Carbonate is used as an inactive ingredient in drug products that have been approved by FDA for otic application (maximum ingredient potency: 0.38%), buccal application (maximum ingredient potency: 145.7 mg), and inhalation exposure (maximum ingredient potency: 4.02%).[22] Calcium Carbonate is also an FDA-approved inactive ingredient in oral drug products (maximum ingredient potency: 4 mg to 550 mg).

Potassium Bicarbonate

Potassium Bicarbonate is used as an inactive ingredient (maximum potency: 1.06 mg to 500 mg) in oral drug products that have been approved by FDA.[22] This ingredient is also an FDA-approved inactive ingredient (maximum potency of 8 mg) in drug products administered via the transmucosal route.

Potassium Carbonate

Potassium Carbonate is used as an inactive ingredient in oral drug products (maximum ingredient potency: up to 27.69 mg) and in topical drug products (maximum ingredient potency not stated).[22]

Toxicokinetic Studies

Absorption, Distribution, Metabolism, and Excretion

Animal

Oral

Magnesium Sulfate

An acid solution of magnesium chloride containing 200 μ curies of magnesium (^{28}Mg) was neutralized with 1 N sodium hydroxide and the precipitate was dissolved in 1 N sulfuric acid.[23] A slightly acidic solution of magnesium sulfate in distilled water (contained 5 meq of magnesium) was fed (in feed) to 3 groups of 6 rabbits (domestic rabbits of mixed breed). The 3 groups were treated as follows: Group 1 (feed and water withheld for 17 h), Group 2 (animals starved for 36 h),

and Group 3 (animals starved for 48 h). In the 3 groups, mean urinary excretion of radioactivity in 48 h ranged between 10% and 12.5%. An external survey of Group 2 animals at 24 h revealed a maximal concentration of radioactivity in the mid abdomen. Two of the animals were killed, and the cecum and its contents were found to contain 78% of the ingested dose. The results of this study indicate that poor gastrointestinal absorption of magnesium accounts for its low renal excretion. Additionally, absorption does not appear to occur from the large intestine.

Intraperitoneal

Calcium Carbonate. Calcium Carbonate (0.40 mCi of calcium [^{14}C]carbonate pellet) was implanted intraperitoneally into a male rat.[24]

Approximately 72% of the radioactivity was excreted as respiratory carbon dioxide between 2 h and 142 h after implantation (most after 69 h). Approximately 30% of the dose was recovered in unabsorbed pellet. Urinary radioactivity accounted for 0.27% of the dose and fecal radioactivity accounted for approximately 0.07% of the dose; 1% of the absorbed dose was retained by the tissues.

Sodium Bicarbonate. Rats (number, strain, and sex not stated) received 5 intraperitoneal (i.p.) injections of [^{11}C]sodium bicarbonate, made at 30-min intervals.[25] The dose/concentration of the test substance was not stated. The animals were killed 30 min after the last injection. Approximately 60% of the radioactivity was accounted for. The urine contained 1.3% of the radioactivity, and >50% of the radioactivity occurred as respiratory [^{11}C]carbon dioxide.

Human

Oral

Calcium Carbonate

The absorbability of calcium from Calcium Carbonate and calcium citrate salts was evaluated in a study in which 37 men and women ingested 300 mg (low load) and 1000 mg (high load) calcium loads as part of a light breakfast meal.[26] The subjects were randomly assigned to a sequence of either Calcium Carbonate first and then calcium citrate, or vice versa. Each absorption measurement was conducted over 2 study days. On the first day, the subjects received no supplement; on the second day, the subjects received a 1000 mg calcium load. The test with the 300 mg load was performed similarly. For the high load, absorption was measured by tracer appearance in the serum and by absorptive increment in urinary calcium. For the low load, absorption was measured using the tracer method only. Test materials (in gelatin capsules) were labeled with ^{45}Ca. Individual tracer doses were in the range of 5 to 9 μCi. Relative absorption was estimated from the difference in urinary calcium following breakfast on the 2 d and true fractional absorption from the appearance of ^{45}Ca in the serum. Mean tracer absorption for both salts combined was 36% at the 300 mg load and 28.4% at the 1000 mg load. In both experiments, the observed mean difference in absorption between the salts was very small. By the tracer method, the within-subject difference (Calcium Carbonate less citrate) was +3.3% ± 1.2% of the ingested dose at the high load and 3.6% ± 2.7% at the low load.

Potassium Bicarbonate

Following ingestion, Potassium Bicarbonate rapidly dissociates in gastric juice to yield carbonate ions (bicarbonate and carbonate) and potassium ions.[27] At this stage, the minor alkalinity is neutralized by the stomach acid. The undissociated Potassium Bicarbonate is not expected to be systemically available in the body under normal handling and use conditions.

Potassium Carbonate

Like Potassium Bicarbonate, following ingestion, Potassium Carbonate rapidly dissociates in the gastric juice to yield carbonate ions and potassium ions.[28] Similarly, the dissociation into ions (in gastric juice) of the following other carbonate salts reviewed in this safety assessment would be expected: Magnesium Carbonate, Ammonium Bicarbonate, Ammonium Carbonate, and Calcium Carbonate.

Toxicological Studies

Acute Toxicity Studies

Dermal

Ammonium Carbonate. The acute dermal toxicity of Ammonium Carbonate was evaluated in accordance with OECD Guideline 402 using 10 (5 male, 5 female) rats of the CRL:(WI) strain.[29] The test substance (200 mg/kg body weight) was applied to 10% of the total body surface for 24 h. The application site was covered with a gauze pad that was secured with a semi-occlusive plastic wrap. Dosing was followed by a 14-d observation period, after which necropsy was performed. None of the animals died and there were no clinical signs of toxicity. Additionally, there were no effects on body weight. It was concluded that the LD_{50} was >2000 mg/kg body weight.

Ammonium Carbamate

The acute dermal toxicity of ammonium carbamate (43.6% ammonium and 56.3% carbon dioxide) was studied using 10 (5 male, 5 female) rats of the Crl:CD(SD) strain.[30] The test substance (in water; 5000 mg/kg weight) was applied, under a semi-occlusive patch to the back for 24 h. Dosing was followed by a 14-d observation period and necropsy. None of the animals died and there were no test substance-related clinical findings. There also were no remarkable body weight changes during the study. At necropsy, scabbing at the application site was observed in 3 animals. The reported LD_{50} was >5000 mg/

kg. Results relating to skin irritation are included in the section on Skin Irritation and Sensitization.

Calcium Carbonate

Calcium Carbonate (nano form) was tested in an acute dermal toxicity study involving 10 (5 male, 5 female) Wistar rats.[26] The test substance was applied to the back and flanks (~10% of total body surface area; 2000 mg/kg body weight) of each animal, and the application site was covered with a semi-occlusive patch for 24 h. The animals were killed at the end of the study and subjected to gross necropsy. None of the animals died and there were no clinical signs of systemic toxicity or dermal irritation. There was no evidence of abnormalities at necropsy. The reported acute dermal LD_{50} was >2000 mg/kg body weight.

Potassium Bicarbonate

The acute dermal toxicity of Potassium Bicarbonate was studied using New Zealand white rabbits (5 males, 5 females).[27] Potassium Bicarbonate was applied to the back at a dose level of 2000 mg/kg body weight, and the application site was covered with a 10 cm x 10 cm occlusive patch. The test substance was applied to the application site at a rate of approximately 0.05 g/cm^2 during a 24-h exposure period. Dosing was followed by a 14-d observation period. All animals were killed and subjected to gross necropsy. None of the animals died and they all appeared clinically normal throughout the study. With the exception of an incidental dermal finding in 1 animal, there were no visible lesions at necropsy. The slight to moderate dermal irritation observed had cleared in all animals by day 10. The dermal LD_{50} was reported to be >2000 mg/kg body weight.

Potassium Carbonate

An acute dermal toxicity evaluation using data on Potassium Carbonate containing a pesticide (identity and concentration of pesticide not stated; concentration of Potassium Carbonate not stated) was performed according to the U.S. EPA pesticide assessment guidelines.[28] The test substance, moistened with distilled water, was applied to the skin of young adult, New Zealand white rabbits (5 males, 5 females [fasted]) for 24 h. The area of application, dose per cm^2, and whether or not the site was covered were not stated. Application of the test substance was followed by a 14-d observation period. None of the animals died. Dermal irritation was observed at the test site; however, irritation scores were not provided. There were no gross findings at necropsy, and neither adverse pharmacologic effects nor abnormal behavior were observed. The reported dermal LD_{50} was >2000 mg/kg body weight for Potassium Carbonate containing a pesticide.

Ammonium Sulfate

The acute dermal toxicity of ammonium sulfate was evaluated according to OECD Guideline 434 using groups of 6 (3 males, 3 females per group) Wistar rats.[25] Ammonium sulfate (in water-acetone solution; single dose of 2000 mg/kg body weight) was applied to a 3 × 4 cm^2 area of the back. The application site was not covered. Dosing was followed by a 14-d observation period. The reported LD_{50} was >2000 mg/kg body weight.

Oral

Ammonium Bicarbonate. An LD_{50} of 1576 mg/kg was reported for rats (number and strain not stated) in an acute oral toxicity study on Ammonium Bicarbonate.[31] Additional study details were not reported.

Ammonium Carbonate

The acute oral toxicity of Ammonium Carbonate (in 0.5% aqueous carboxymethyl cellulose) was studied using groups of 5 male and 5 female Wistar rats.[25] Ammonium Carbonate was administered to 4 groups at the following oral doses, respectively: 215 mg/kg, 681 mg/kg, 1470 mg/kg, and 2150 mg/kg. The maximum dose volume per group was 10 ml. Untreated rats served as controls. Dosing was followed by a 14-d non-treatment period. Five rats dosed with 2150 mg/kg and 3 rats dosed with 1470 mg/kg died. There were no mortalities in the 2 lower dose groups. Except for paresis, observed only in male rats (1470 mg/kg dose group), the following clinical signs were observed in male and female rats (number affected not stated) of the 1470 mg/kg dose group prior to death: dyspnea, apathy, abnormal position, staggering, tonic convulsions, exophthalmos, salivation, and poor general state. Necropsy findings in animals that died included general congestion of the glandular stomach (mucosa slightly red). There were no pathological findings in animals that were killed. An LD_{50} of 1576 mg/kg was reported for male/female rats.

Ammonium Carbamate

Groups of male and female rats (1 to 5/group) were dosed orally (by gavage) with ammonium carbamate at up to 4000 mg/kg body weight.[30] The 800, 1000, and 2000 mg/kg body weight groups consisted of 5 animals each; the remaining groups consisted of 1 animal per group. Deaths occurred within 30 min of exposure, and the clinical signs observed included apathy, convulsions, and accelerated respiration. Gross pathology data were not available. An LD_{50} of 1380 mg/kg body weight was reported.

Calcium Carbonate

Calcium Carbonate (in arachis oil) was administered to 5 female Sprague-Dawley rats at a single oral dose of 2000 mg/kg body weight.[26] The dose volume was 10 ml/kg. Dosing was followed by a 14-d observation period and necropsy. None of the animals died and there were no clinical signs of systemic toxicity. Additionally, there were no adverse changes in body

weight, and adverse effects were not observed at necropsy. The reported LD_{50} was >2000 mg/kg body weight.

Potassium Bicarbonate

A single oral dose of Potassium Bicarbonate (in distilled water; 2000 mg/kg body weight) was administered by gavage to Sprague-Dawley rats (5 males, 5 females).[27] Dosing was followed by a 14-d observation period and necropsy. None of the animals died. Except for piloerection observed in all animals during the first 30 min post-application, there were no treatment-related clinical signs or changes in body weight. There also were no treatment-related necropsy findings. Potassium Bicarbonate was classified as non-toxic and the reported LD_{50} was >2000 mg/kg body weight.

Potassium Carbonate

In an acute oral toxicity study using rats (number and strain not stated), a mean LD_{50} of 1870 (range: 1340 to 2600) mg/kg was reported after gastric intubation with Potassium Carbonate (0.20 g/ml).[32] Additional study details were not presented.

In a study summarized in a European Union; Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossier on Potassium Carbonate, "potash calc." (composition not stated) was evaluated in an acute oral toxicity study using a procedure that was equivalent to that of the now discontinued OECD Guideline 401.[28] Fasted Sprague-Dawley rats (5 males, 5 females) were dosed with the test substance; dosing was followed by a 14-d observation period. None of the animals died, and there were no treatment-related clinical signs, necropsy findings, or changes in body weight. The LD_{50} was >2000 mg/kg body weight.

Inhalation

Ammonium Bicarbonate and Ammonia. The acute inhalation toxicity of ammonia was studied using 3 groups of male ICR mice (number per group not stated).[30] The 3 groups were exposed for 1 h to ammonia (method not stated) at the following concentrations: 2408 mg/m^3 (3440 ppm), 2954 mg/m^3 (4220 ppm), and 3402 mg/m^3 (4860 ppm). Dosing was followed by a 14-d observation period and necropsy. The lungs of mice that died (number not stated) were diffusely hemorrhagic. Acute vascular congestion and diffuse intra-alveolar hemorrhage were observed microscopically. Mild to moderate chronic focal pneumonitis was also observed. In animals that survived, focal atelectasis and liver damage were observed in animals that were killed after the observation period. Other findings in surviving animals included: swelling and increased cytoplasmic granularity of hepatocytes (at 3340 ppm), scattered foci of frank cellular necrosis of hepatocytes (at 4220 ppm), increased necrosis of hepatocytes (at 4860 ppm), and follicular hyperplasia in the spleen (test concentrations not stated). This finding for the spleen was not observed in animals that died during exposure. It was noted that the liver lesions may have resulted from the compromised nutritional state of the mice. The LC_{50} for Ammonium Bicarbonate was deduced using the maximum quantity of NH_3 possibly released from ammonium carbamate (i.e., 43.6%). The calculated LC_{50} values for Ammonium Bicarbonate (in mice) were: 6.8 mg/l air (for 1 h exposure) and 1.7 mg/l air (4534 ppm [extrapolated using Haber's law]).

Calcium Carbonate

The acute inhalation toxicity of Calcium Carbonate was evaluated according to OECD Guideline 403 using 10 (5 males, 5 females) Wistar rats.[26] The animals were exposed for 4 h to aerosolized Calcium Carbonate using a nose-only exposure system. Exposure was interrupted for a total of 8 min. The flow of air in each tube was 0.97 l/min, and the mean chemical aerosol concentration was 3 mg/l of air. Mass mean aerodynamic diameters (MMAD) were between 2.28 µm and 2.89 µm, with a geometric standard deviation between 1.5 and 3. Taking these values into consideration, it was assumed that the deposition of particles would occur in the upper and lower respiratory tract. Exposure was followed by a 14-d observation period. All animals were killed and subjected to gross necropsy. Ruffled fur was the only clinical sign that was observed. Slight body weight loss, followed by recovery, was observed. There were no macroscopic findings at necropsy. The reported LC_{50} was >3 mg/l of air.

Potassium Bicarbonate

The acute inhalation toxicity of Potassium Bicarbonate was evaluated using Sprague-Dawley rats (5 males, 5 females).[27] The animals were exposed for 45.5 h to aerosolized Potassium Bicarbonate at a mean gravimetric chamber concentration of 4.88 ± 0.6 mg/l. Approximately 1% of the particles were of a size <1 µm; 25% were <3 µm. The mass mean aerodynamic diameter was ~4.7 µm. The following signs were observed during the first hour of exposure: decreased activity, ocular discharge, and hunched posture. Similar signs as well as facial staining and/or nasal discharge were observed after removal of the animals from the exposure chamber. All of the animals recovered from these signs within 24 h. Gross necropsy findings were unremarkable, and all tissues and organs appeared normal. The LC_{50} was reported to be >4.88 ± 0.60 mg/l air.

Potassium Carbonate

In a REACH dossier on Potassium Carbonate, an acute inhalation toxicity evaluation using data on Potassium Carbonate containing a pesticide (identity and concentration of pesticide not stated; concentration of Potassium Carbonate not stated) was performed according to the US EPA pesticide assessment guidelines.[28] Sprague-Dawley rats (5 males, 5

females) were exposed to the aerosolized test substance (mass mean aerodynamic diameter ≈3.6 μ) for 4.5 h. The gravimetric chamber concentration was 4.96 ± 1.14 mg/l, with approximately 3% of the particles below 1 μm, and 38% below 3 μm. Exposure was followed by a 14-d observation period. None of the animals died. Dermal necrosis and corneal opacity were observed in all animals, and damage was most severe around the mouth and on the forelimbs. There were no test substance-related gross necropsy findings. The LC_{50} was >4.96 ± 1.14 mg/l air.

Sodium Bicarbonate

The acute inhalation toxicity of sodium bicarbonate was evaluated using groups of 10 (5 males, 5 females per group) Sprague-Dawley rats.[25] The mass mean aerodynamic diameter of the test substance was 2.9 ± 1.77 μm. The animals were exposed to aerosolized sodium bicarbonate (4.74 ± 1.03 mg/l) for 4.5 h, followed by a 14-d observation period. None of the animals died, and necropsy was performed at the end of the observation period. Ocular/nasal discharge was observed in 6 of 10 rats. Moderately red lung tissue was reported for 1 male and 1 female. One male had slightly red lung tissue. The necropsy findings were classified as unremarkable. The reported LC_{50} was >4.74 mg/l air.

Intravenous

Ammonium Bicarbonate. The acute intravenous (i.v.) toxicity of Ammonium Bicarbonate (in 0.03 M sodium hydroxide) was evaluated using groups of 10 young albino mice, and a mean LD_{50} value of 3.10 ± 0.28 mM/kg body weight was reported.[33] Additional study details were not included.

Ammonium Bicarbonate

and Ammonium Carbamate. The acute i. v. toxicity of an ammonium carbamate/Ammonium Bicarbonate mixture (in 0.03 M sodium hydroxide) was evaluated using groups of 10 young albino mice.[33] The test substance was defined as commercial reagent grade Ammonium Carbonate, and was described as a mixture of approximately equal parts Ammonium Bicarbonate and ammonium carbamate. A mean LD_{50} value of 1.02 ± 0.11 mM/kg body weight was reported. Additional study details were not provided.

Short-Term Toxicity Studies

Oral

Magnesium Chloride Hexahydrate. The repeated dose toxicity of magnesium chloride hexahydrate was studied using groups of Wistar rats.[23] The dose groups were as follows: 250 mg/kg body weight/day (10 males, 10 females), 500 mg/kg body weight/d (12 males, 12 females), and 1000 mg/kg body weight/d (15 males, 15 females). The vehicle (water) control group consisted of 12 males and 12 females. The test substance was administered orally (by gavage) to males and females daily during 14 d pre-mating and 14 d of mating. The test substance was also administered to females during gestation and up to postnatal day (PND) 3, and to males for 28 to 29 d. There were no treatment-related mortalities or major toxicological findings. There were no remarkable clinical signs or effects on hematology or clinical chemistry. The same was true for necropsy and histopathological findings relating to non-reproductive organs. It was concluded that the NOAEL for magnesium chloride hexahydrate was 1000 mg/kg body weight/d. Furthermore, it was determined that the equivalent NOAEL for Magnesium Carbonate was 414 mg/kg body weight/d. Results relating to toxic effects of magnesium chloride hexahydrate on reproductive organs are included in the section on Developmental and Reproductive Toxicity.

Calcium Carbonate

Five rats were fed [^{45}Ca]labeled Calcium Carbonate (0.3 g/kg body weight) in feed for 3 d.[24] The strain of rats tested was not identified. All of the animals remained healthy.

The oral toxicity of Calcium Carbonate (nano form) was evaluated in a 14-d study using groups of 6 (3 male, 3 female) Wistar rats.[26] The 4 groups consisted of the control and 250, 500, and 1000 mg/kg bodyweight/d groups. The test substance was administered once daily by gavage at a dosage volume of 5 ml/kg. Control animals were dosed with distilled water. There were no unscheduled deaths or treatment-related toxicologically significant macroscopic abnormalities. There also were no clinical signs of toxicity or adverse effects on body weight development. The NOAEL was 1000 mg/kg body weight/d.

Potassium Bicarbonate

In a 4-wk toxicity study, groups of 10 male and 10 female SPF-bred Wistar rats (CpB:WU;Wistar random) were fed unsupplemented rodent diet (control) or a diet containing 2% or 4% Potassium Bicarbonate.[34] The animals were killed at the end of the study. None of the animals died during the study, and no treatment-related abnormalities were reported. There were no consistent or treatment-related effects on red blood cell variables, clotting potential, or total and differential white blood cell counts in any of the groups. Relative kidney weights (relative to body weight) were increased; however, this finding was not consistent or considered to be dose-related. At necropsy, macroscopic examination did not reveal any significant differences among test and control groups, except for macroscopic lesions in the urinary bladder of some rats. Most of the histopathological changes observed represented background pathology that is normal for SPF-bred Wistar rats (CpB:WU; Wistar random).

Ammonium Chloride

The oral toxicity of ammonium chloride was evaluated in a 28-d study involving groups of 10 male and female Wistar rats.[25]

Ammonium chloride was fed at a concentration of 2% (2214.5 mg/kg body weight/d) or 4% (4228.5 mg/kg body weight/d) in the diet daily for 28 d. The animals were killed at the end of the study and necropsied. Organs were subjected to microscopic examination. Significant weight loss (18% to 25% below the control value; $P < .01$) was noted for males and females of the higher dose group; food intake was decreased. Growth was also markedly decreased ($P < .05$) in the lower dose group. None of the animals died and there were no treatment-related clinical signs. Urinalyses revealed a dose-dependent increase in net acid excretion. There were no consistent or treatment-related defects on red blood cell variables, clotting potential, or total and differential white blood cell counts. Alkaline phosphatase activity was increased in the higher dose group (27.7% over controls, both sexes) and in low-dose males (27.7% over controls). There were no consistent or treatment-related changes in the following organ weights: liver, spleen, ovaries, pituitary, thyroid, thymus, or heart. Relative kidney weight (relative to body weight) was increased in both dose groups. Relative adrenal weight was increased only in males of the higher dose group. There were no significant differences in necropsy findings between test and control groups. Most of the histopathological changes observed were nearly equally distributed among control and test groups, and represented normal background pathology for rats of this strain and age. It was noted that most of the changes could be regarded as physiological adaptations to the feeding of acid-forming salt.

Ammonium chloride was fed (in the diet) to10 male Sprague-Dawley rats at 684 mg/kg body weight/d (7 d/wk) for 70 d.[30] Negative and positive control (tributyl phosphate) groups were included in the study. A 7-d post-exposure period was observed, and gross and microscopic examinations of the bladder were performed. There were no significant differences in food consumption among the 3 groups. The difference also was not significant when animals fed ammonium chloride were compared to the negative control group. Body weight was significantly decreased in the positive control group. Gross and microscopic examination results were negative for animals fed ammonium chloride and for the negative control group. Hyperplasia of the bladder was observed in the positive control group. The NOEL for ammonium chloride was <684 mg/kg body weight/d.

Inhalation

Potassium Carbonate. The potential for short-term toxicity and neurotoxicity of a Potassium Carbonate-based scrubbing solution (containing 30.8% (w/v) Potassium Carbonate) used in petroleum refineries was evaluated in Sprague-Dawley Crl: CD BR rats.[35] Inhalation exposures were to aerosols of a "used" scrubbing solution in a whole-body exposure chamber, 6 h/d for 21 consecutive days at target concentrations of 0 (filtered air—control), 0.1, 0.2, or 0.4 mg/l (30 animals/sex/group). Five rats per sex per group were allowed a 14-d recovery period (satellite recovery group) and killed on study day 35 for either systemic or neurotoxic evaluation. Functional observation battery examinations and locomotor activity tests were conducted. No apparent adverse effects were noted at any exposure level, as determined by clinical observations, food consumption measurements, hematology, serum chemistry, ophthalmologic observations, and gross pathology evaluations. Statistically significant increases in lung weights were noted at all concentrations; all lung weights returned to control values at the end of exposure, except for the 0.4 mg/l group (females). There were no significant changes in other organ weights. Histopathologic findings were restricted to the respiratory tract and were characterized by minimal to moderate epithelial hyperplasia, epithelial necrosis, and cytoplasmic vacuolation at levels I and II of the nasal cavities. The mild cytoplasmic vacuolization of the olfactory epithelium was observed in the 0.2 and 0.4 mg/l exposure groups. Minimal epithelial necrosis at level II of the nasal cavities was observed in the 0.4 mg/l exposure group. Lung bronchiolization and alveolar macrophage infiltration were also observed in the 0.2 and 0.4 mg/l exposure groups. The respiratory tract findings were considered a local response to the high alkalinity of the test material, as substantiated by the return to normal upon cessation of exposure. Exposure to the scrubbing solution had no adverse effect on functional observation battery endpoints or locomotor activity, brain weight and size, and neuropathologic assessments. The authors concluded that inhalation exposure to a Potassium Carbonate-based scrubbing solution aerosol for 21 d did not result in any persistent systemic toxicity, including neurotoxicity, in male or female rats.

Subchronic Toxicity Studies

Oral

Potassium Bicarbonate. In a 13-wk toxicity study, groups of 10 male and 10 female SPF-bred Wistar rats (CpB:WU;Wistar random) were fed unsupplemented rodent diet or a diet containing 2% or 4% Potassium Bicarbonate.[34] The results reported in this study were identical to those stated in the 4-wk study on Potassium Bicarbonate (in the Short-term Oral Toxicity section), except for the following: Zona glomerulosa hypertrophy (classified as a non-neoplastic histopathological change) was observed at a concentration of 4%, and this finding was statistically significant ($P < .01$) compared to the control. The finding of oncocytic kidney tubules (also classified as non-neoplastic histopathological change) was statistically significant, compared to the control, at a concentration of 4% ($P < .01$).

The effect of Potassium Bicarbonate (2% or 4%) on rat urinary bladder epithelium was studied without prior exposure to a bladder tumor initiator.[36] In 4 studies, ranging in duration from 4 to 130 weeks, equimolar amounts of K+ were administered in the diet to male and female weanling, SPF-bred Wistar rats (Cpb:WU; Wistar random) (85 rats/sex/group) as

Potassium Bicarbonate. Increased urinary volume and potassium levels were observed, and urinary pH was increased. Results relating to carcinogenicity are included in the Carcinogenicity section.

Groups of 10 weanling male SPF-bred albino rats (Cpb: WU; Wistar random) were fed a basal diet or diet supplemented with Potassium Bicarbonate (2.5% in the diet) for up to 13 weeks.[37] A group of 10 rats was also fed 6% monosodium glutamate (MSG) in the diet. Feeding with MSG induced slight growth retardation, decreased food intake (mainly with the purified diet), and increased kidney-to-body weight ratios. The addition to stock diet of 2.5% Potassium Bicarbonate, instead of MSG, induced changes in growth rate, food intake, and kidney weight that were similar to those observed with 6% MSG. Results relating to carcinogenicity are included in the Carcinogenicity section.

Another 13-wk study was performed using groups of 10 weanling male SPF-bred albino rats (Cpb: WU; Wistar random) to compare the effects of 5% Potassium Bicarbonate in a stock diet and in a purified diet.[37] Unsupplemented stock and purified diets served as controls. The rats were gradually accustomed to the high level of Potassium Bicarbonate (5%) by feeding 1% in the diet during week 1, 2% in the diet during week 2, 3% in the diet during week 3, 4% in the diet during weeks 4 and 5, and 5% in the diet from week 6 on. Growth was retarded by 5% Potassium Bicarbonate in the groups on either basal diet; the difference was not statistically significant for the rats fed 5% Potassium Carbonate in the stock diet, but it was statistically significant in the rats fed 5% Potassium Carbonate in the purified diet. None of the rats had any abnormalities in condition or behavior. At microscopic examination, there were no treatment-related changes in the following organs: ureters, kidneys, liver, testes, thyroid with parathyroids, and adrenals. Results relating to hyperplastic changes in the bladder epithelium are included in the Carcinogenicity section.

Chronic Toxicity Studies

Oral

Potassium Bicarbonate. In an 18-month toxicity study, groups of 15 male and 15 female SPF-bred Wistar rats (CpB: WU;Wistar random) were fed unsupplemented rodent diet or a diet containing 2% or 4% Potassium Bicarbonate.[34] The animals were killed at the end of the study. There were no consistent or treatment-related effects on red blood cell variables, clotting potential, or total and differential white blood cell counts in any of the groups. At necropsy, macroscopic examination did not reveal any significant differences among test and control groups, except for macroscopic lesions in the urinary bladder of some rats. Most histopathological changes observed were considered equally distributed among the treatment groups and the controls, and represented normal background pathology for rats of this strain and age. The following statistically significant changes (compared to the control) were observed: zona glomerulosa hypertrophy (classified as a non-neoplastic histopathological change) at a concentration in feed of 4% ($P < .01$); oncocytic kidney tubules (classified as a non-neoplastic histopathological change) at a concentration of 2% ($P < .05$) and 4% ($P < .01$); simple urothelial hyperplasia of the urinary bladder (classified as a non-neoplastic histopathological change) at concentrations of 2% and 4% ($P < .05$); and papillary/nodular hyperplasia of the urinary bladder (classified as a non-neoplastic histopathological change) at a concentration of 2% ($P < .01$). The papillary/nodular hyperplasia of the urinary bladder observed in the 4% dietary group was not statistically significant.

In a 30-month carcinogenicity study, groups of 50 SPF-bred weanling Wistar rats (CpB:WU;Wistar random) per sex were fed a natural ingredient diet (controls) or diet supplemented with 2% or 4% Potassium Bicarbonate.[34] There were no treatment-related mortalities. At necropsy, macroscopic examination did not reveal any significant differences among test and control groups, except for macroscopic lesions in the urinary bladder of some rats. Most histopathological changes observed were considered equally distributed among the treatment groups and the controls and represented normal background pathology for rats of this strain and age. Results relating to carcinogenicity are included in the Carcinogenicity section.

Developmental and Reproductive Toxicity Studies

Oral

Magnesium Chloride Hexahydrate. The reproductive and developmental toxicity of magnesium chloride hexahydrate was studied using groups of Wistar rats.[23] The dose groups were as follows: 250 mg/kg body weight/d (10 males, 10 females), 500 mg/kg body weight/d (12 males, 12 females), and 1000 mg/kg body weight/d (15 males, 15 females). The vehicle (water) control group consisted of 12 males and 12 females. The test substance was administered orally (by gavage) to males and females daily for 14 d pre-mating and 14 d of mating. The test substance was also administered to females during gestation and up to PND 3, and to males for 28 to 29 d. No test substance-related histopathological lesions were observed in the reproductive organs of male or female rats dosed with the test substance (all dose groups). There were no treatment-related effects with respect to the following when compared to controls: mean number of corpora lutea, number of implantation sites, total number of pups born, number of males, number of females, sex ratio, live pups, stillbirth, runt on PND 0, and total number of live pups and sex ratio on PND 4, pre-implantation loss and post-implantation loss. The survival of pups (in all treatment groups) from PND 0 to PND 4 was not affected by treatment. At necropsy, there were no gross external abnormalities in pups from any dose group. It was concluded that the NOAEL for reproductive/developmental toxicity of magnesium chloride hexahydrate

was 1000 mg/kg body weight. Furthermore, it was determined that the equivalent NOAEL for Magnesium Carbonate was 414 mg/kg body weight/d. Additional results from this study are included in the Short-Term Oral Toxicity section of this report.

The teratogenicity of magnesium chloride hexahydrate (in distilled water) was evaluated using groups of 22 pregnant female Wistar rats.[23] The 3 dose groups received oral doses (by gavage) of 200, 400, and 800 mg/kg/d, respectively, on gestation days 6 to 15 (10 d). A fourth group served as the control. The animals were killed on day 20 of pregnancy. Regarding maternal toxicity, there were no clinical signs or deaths. Dosing (all groups) did not cause increased incidences of the following: number of implantations, number of corpora lutea, % implantation loss, number of offspring alive, sex ratio of offspring, offspring weight or embryo/intrauterine fetal death. Gross malformation was observed in the dose groups, but without any intergroup differences. Bone malformation was observed in one fetus of the 800 mg/kg/d dose group. There were no intergroup differences in bone abnormality, effects on lumbocostale, extra ribs, sacrococcygea, metacarpal bone, or its ossification. Visceral malformations were observed in 4 to 6 fetuses from each dose group, but without intergroup differences. It was concluded that magnesium chloride hexahydrate was not teratogenic in rats dosed by gavage. The NOAEL was estimated to be >800 mg/kg body weight/d for pregnant rats and their fetuses. The equivalent NOAEL for Magnesium Carbonate was determined to be >331 mg/kg body weight/d.

Calcium Carbonate

Female Swiss mice were bred after being fed (number of animals/feeding duration not stated) a diet supplemented with 0.5%, 1%, or 2% Calcium Carbonate.[24] First and second litters were studied. Calcium Carbonate (1% and 2% in diet) yielded an intake of approximately 3000 mg/kg body weight. When compared to the control diet, feeding with the supplemented diet significantly decreased the number and total weight of the weanling mice, and increased the proportion of deaths. Calcium Carbonate (2%) in the diet also caused hypertrophy of the heart and a tendency toward decreased thymus weight in weanling mice.

The reproductive toxicity of Calcium Carbonate (nano form) was studied using groups of 20 (10 male, 10 female) Wistar rats.[26] Three groups received oral dosage rates of 100, 300, and 1000 mg/kg body weight/day, respectively. The doses were administered daily for 48 consecutive days (including a 2-wk maturation phase, pairing, gestation, and early lactation). Untreated animals served as controls. The dosage volume was 5 ml/kg. Males were killed on day 43, and females were killed on day 5 post-partum. Gross pathology and histopathology were performed. There were no test substance-related mortalities or toxicologically significant macroscopic or microscopic findings in parental animals. All offspring were subjected to a full external and internal examination; any macroscopic abnormalities were recorded. There were no test substance-related effects on reproductive performance and length of gestation. When compared to controls, there were no significant differences with respect to corpora lutea and implantation counts. Litter sizes and viability for treated groups were also comparable to controls. There also were no obvious clinical signs of toxicity in offspring from treated females, or test substance-related gross pathological findings in offspring. Because there were no treatment-related effects on reproduction, the NOEL for reproductive toxicity was considered to be 1000 mg/kg body weight/d.

In a 62-d developmental toxicity study involving 4 groups of female Charles River CD/VAF Plus rats (mated with male rats), Calcium Carbonate (in the diet) was fed at concentrations of 0.05% (control), 0.75%, 1%, and 1.25% for 6 wk prior to mating, during mating, and for 20 d of gestation.[26] For each dose group, the number of females dosed prior to mating was 69 and the number of pregnant rats dosed (through mating to gestation day 20) ranged from 45 to 48. The male to female ratio per cage was 1:2. Male rats were dosed only during the mating period. On gestation day 20, the animals were killed, and Cesarean sections were performed. There was no evidence of test substance-related maternal toxicity or embryotoxic/teratogenic effects. There were no dose-related changes in the average number of implantations, resorptions and viable fetuses, or fetal length or weight. When compared to the control group, there were no statistically significant increases in the litter incidence regarding specific external, visceral, or skeletal variations of the fetuses. The NOAEC for teratogenicity was >1.25% Calcium Carbonate in the diet and corresponded to a NOAEL between 1963 and 2188 mg/kg body weight per day.

Potassium Carbonate

The teratogenicity of Potassium Carbonate was evaluated using groups of 22 to 25 CD-1 mice, according to a protocol similar to OECD Test Guideline 414.[28,38] The test substance was administered, by gavage, at doses of 0, 2.9, 13.5, 62.5, or 290 mg/kg body weight/d, on gestation days 6 through 15. On day 17, Caesarean section was performed on all of the dams, and the following information was recorded: sex, numbers of corpora lutea, implantation sites, resorption sites, live and dead fetuses, and body weights of live pups. The urogenital tract of each dam was examined in detail for anatomical normality. All of the fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses in each litter were subjected to detailed visceral examinations, and the remaining two-thirds were examined for skeletal defects. There were no effects on mortality, body weight gain, or the urogenital tracts of dams. The no-observed-effect-level (NOEL) for maternal toxicity was 290 mg/kg body weight/d (i.e., the highest dose tested). There were no effects on any of the following: numbers of corpora

lutea, live litters, implantations, resorptions, live and dead fetuses, the sex ratio of the fetuses, or the average fetal weight. The incidence of soft tissue and skeletal abnormalities within groups treated with Potassium Carbonate did not differ from that of sham-treated controls. The NOEL for developmental toxicity/teratogenicity was 290 mg/kg body weight/d.

The teratogenicity of Potassium Carbonate was also evaluated using groups of 22 to 25 albino rats (Wistar-derived stock).[38] The test substance was administered (by oral intubation), on gestation days 6 through 15, at dose rates of 0, 1.8, 8.4, 38.8, or 180 mg/kg body weight/d according to the procedure in the preceding experiment, except that Caesarean section was performed on day 20. There were no discernible effects on nidation or on maternal or fetal survival. Furthermore, the number of abnormalities observed in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.

Sodium Bicarbonate

The embryotoxicity/teratogenicity of sodium bicarbonate was evaluated using groups of 25 female Wistar rats.[25] After mating, groups of female rats were dosed orally (intubation) with sodium bicarbonate (in water) on gestation days 6 through 15. The following dosages were administered to the 4 groups, respectively: 3.4, 15.8, 73.3, and 340 mg/kg body weight. Two additional groups were sham-exposed and dosed with aspirin (positive control), respectively. The animals were killed on gestation day 17. There was no evidence of embryotoxicity or teratogenicity. The number of abnormalities observed in either soft or skeletal tissues of test animals did not differ from the number occurring spontaneously in sham-treated controls.

The embryotoxicity/teratogenicity of sodium bicarbonate was evaluated using groups of nulliparous female Sprague-Dawley rats of the Crj:CD(SD) strain.[29] After mating was confirmed (designated as gestation day 0), the animals were dosed orally with 2% sodium bicarbonate (in drinking water) on gestation days 15 through 20. Two groups were dosed orally (by gavage) with 0.5% aqueous methylcellulose on day 16 of gestation and were given either tap water (control group) or 2% sodium bicarbonate solution in drinking water. An untreated group served as the concurrent control. The animals were killed on gestation day 20 and fetal external examinations performed. Dosing with sodium bicarbonate had no effect on the number of implants, % resorptions, or the number of live fetuses per litter. The average body weights of the live fetuses of pregnant females treated with sodium bicarbonate were comparable to those of the control group. There were no treatment-related abnormalities in groups treated with sodium bicarbonate.

The developmental toxicity of sodium bicarbonate was studied using groups of 50 (25 males, 25 females/group) CD-1 mice.[30] Four groups were dosed orally (by gavage) with sodium bicarbonate at 5.8 mg/kg body weight, 27 mg/kg body weight, 125 mg/kg body weight, and 580 mg/kg body weight, respectively, on days 6 through 15 of gestation (17 d). A fifth group served as the sham-treated control. The number of abnormalities observed in soft or skeletal tissues of the treatment groups did not differ from the number occurring spontaneously in the sham-treated controls.

Inhalation

Potassium Carbonate. The developmental toxicity potential of a scrubbing solution containing 30.8% Potassium Carbonate, used extensively in petroleum refineries to remove carbon dioxide from hydrogen gas streams, was evaluated.[39] Pregnant female CD (Sprague-Dawley) rats (number not stated) were exposed to aerosols of a "used" scrubbing solution at 0.05, 0.1, 0.2, or 0.3 mg/l for 6 h/d on days 6–19 of pregnancy. Control animals were exposed to filtered air under the same exposure conditions. Dams were killed on day 20 of pregnancy and a laparohysterectomy was performed. The mass median aerodynamic diameter of aerosol particles ranged from 1.6 to 2.8 μm, with geometric standard deviations between 2.0 and 2.3 μm. The overall pregnancy rate was high (>95%) and equivalent across all groups. All pregnant dams had live litters, and 22–24 litters were examined in each group. Treatment-related clinical signs consisted of rales, observed at all exposure levels, and gasping only at the 0.3 mg/l exposure level. The occurrence of rales was presumably a localized effect on the respiratory tract and was likely due to the irritating properties of the scrubbing solution. Maternal toxicity was exhibited in the 0.3 mg/l group, including reduced body weight, weight gain, and food consumption, and one possible treatment-related death on gestation day 17. At the scheduled necropsy, there were no treatment-related, gross pathological observations and no statistically significant differences in measurements of reproductive and developmental parameters. The incidences of fetuses with skeletal variations involving the sternum were clustered in two litters at the highest exposure level, with atypically low-term fetal body weights. Under the conditions of this investigation, Potassium Carbonate scrubbing solution was not a developmental toxicant.

Genotoxicity Studies

In Vitro

Ammonium Carbamate. Ammonium Carbonate is a mixture of Ammonium Bicarbonate and Ammonium Carbamate. The genotoxicity of ammonium carbamate was evaluated in the Ames test at concentrations up to 5000 μg/plate with and without metabolic activation.[30] The positive controls were as follows: 4-nitroquinoline-*N*-oxide, 9-aminoacridine, 2-aminoanthracene (2-AA), *N*-methyl-*N*′-nitroso-*N*-nitrosoguanidine (MNNG), and 4-nitro-*o*-phenylenediamine. Ammonium carbamate was not genotoxic.

Calcium Carbonate

The genotoxicity of Calcium Carbonate (nano form) was studied using the mammalian chromosome aberration test, performed in accordance with OECD Guideline 473.[26] Human lymphocytes were incubated with the test substance at concentrations up to 1000 µg/ml with and without metabolic activation. Cyclophosphamide and mitomycin C served as positive controls. Calcium Carbonate (nano form) did not cause a statistically significant increase in the frequency of cells with chromosome aberrations either with or without metabolic activation. The test substance was classified as non-clastogenic.

Potassium Bicarbonate

The genotoxicity of Potassium Bicarbonate was evaluated in the Ames test (with and without metabolic activation) using *Salmonella typhimurium (S. typhimurium)* strains TA1535, TA1537, TA1538, TA98, and TA100, and *Saccharomyces cerevisiae* strain D4.[40] Potassium Bicarbonate was tested at concentrations up to 0.1580% in bacteria and at concentrations up to 3.3% in yeast. Test results were negative with and without metabolic activation, and Potassium Bicarbonate was classified as non-genotoxic.

Potassium Carbonate

In the Ames test, Potassium Carbonate was not genotoxic in *Saccharomyces cerevisiae* strain D4 (yeast) or in the following bacterial strains with or without metabolic activation: *S typhimurium* strains: TA1535, TA1537, and TA1538. Details relating to the test procedure were not included.[24]

Potassium Chloride

The following data on potassium chloride (surrogate compound) are included in a REACH dossier on Potassium Carbonate. The genotoxicity of potassium chloride was evaluated in the L5178Y mouse lymphoma cell mutagenesis assay, at concentrations up to 5000 µg/ml with and without metabolic activation.[28,41] Ethyl methanesulfonate and 3-methylcholanthrene served as positive controls. Results were negative with and without metabolic activation. The positive controls were genotoxic.

Ammonium Sulfate

The genotoxicity of ammonium sulfate (in acetone) was evaluated in the mammalian cell gene mutation assay using V79 Chinese hamster fibroblasts.[25] The test procedure was consistent with OECD Guideline 476. Ammonium sulfate was tested at concentrations up to 1320 µg/ml with and without metabolic activation. Results were negative with and without metabolic activation.

Magnesium Chloride

Magnesium chloride was evaluated in the mammalian chromosome aberration test, without metabolic activation, in accordance with OECD Guideline 473.[23] The test substance (in physiological saline) was evaluated at doses up to 2 mg/ml in Chinese hamster lung fibroblast (V79) cultures incubated for up to 48 h. Untreated and vehicle-treated cultures served as controls. The incidence of polyploid cells at 48 h was 1%, and the incidence of cells with structural chromosomal aberrations at 24 h was 2%. It was concluded that magnesium chloride was non-genotoxic in this assay.

In Vivo

Ammonium Chloride. The genotoxicity of ammonium chloride (in saline) was evaluated in the micronucleus test.[25] Groups of 6 male mice of the ddY strain received i. p. doses up to 500 mg/kg body weight. Control mice were injected i. p. with mitomycin C. Bone marrow from the femur was analyzed. One thousand polychromatic erythrocytes per mouse were scored and the number of micronucleated erythrocytes was recorded. Ammonium chloride was not genotoxic in this assay.

Anti-Genotoxicity Studies

In Vitro

Magnesium Carbonate. The anti-genotoxicity of Magnesium Carbonate in the presence of hydrogen peroxide was evaluated in the Ames test using *S. typhimurium* strain 102.[42] Magnesium Carbonate was tested at a concentration of 25 mM or 50 mM, and each concentration was tested in the presence of 82 mM or 164 mM hydrogen peroxide. Magnesium Carbonate did not cause a decrease in the number of revertants induced by hydrogen peroxide. The number of revertants induced by hydrogen peroxide (164 mM) alone was 695.50 ± 62.7. The combination of Magnesium Carbonate (50 mM) + hydrogen peroxide (164 mM) yielded 746 ± 202 revertants. A control value of 334.20 ± 47.98 revertants was reported.

Magnesium Carbonate was also evaluated for anti-genotoxicity at concentrations of 50 mM and 100 mM (in the presence of hydrogen peroxide) in the suspension test using strain D7 of *Saccharomyces cerevisiae*.[42] Both stationary and logarithmic phase cells were used. The high concentration of Magnesium Carbonate (100 mM) was found to be cytotoxic only in cells in the logarithmic growth phase. Magnesium Carbonate significantly decreased the gene conversion frequency that was induced by 200 mM hydrogen peroxide. Also, the point reverse mutations induced by 200 mM and 400 mM hydrogen peroxide were statistically significantly decreased in the presence of Magnesium Carbonate. In the logarithmic growth phase, Magnesium Carbonate caused a significant

decrease in the gene conversion frequency that was induced by 50 mM and 100 mM hydrogen peroxide. The anti-genotoxic effect of Magnesium Carbonate was not found to be dose-dependent.

The effects of Magnesium Carbonate on the genotoxicity induced by nickel subsulfide were examined using Chinese hamster ovary (CHO) cells and BALB/3T3 fibroblast cells.[42] The cells were incubated, with and without nickel subsulfide (at 1 μg/ml), in the presence of various concentrations of Magnesium Carbonate (0.6, 1.2, 2.4 μg/ml) to give final molar ratios of 0.25, 0.5, and 1.0. The suppression of up to 64% of the proliferation of BALB/3T3 fibroblasts by nickel subsulfide (1 μg/ml) was reversed by Magnesium Carbonate, having recovered slowly in a dose-dependent manner. The nickel compound increased not only the number of micronuclei, but also the number of DNA-protein cross-links examined with CHO and BALB/3T3 cells, respectively. These genotoxic effects of nickel were again lessened by Magnesium Carbonate. The nickel subsulfide at 1 μg/ml increased the number of micronuclei from 12 to 54 in controls, out of 500 binucleated cells. This number was reduced to 34 upon Magnesium Carbonate co-treatment at 2.4 μg/ml. The DNA-protein cross-links coefficient of 1.63 obtained in the presence of nickel was decreased to 1.39 with Magnesium Carbonate co-treatment at 2.4 μg/ml.

Carcinogenicity Studies

Oral

Potassium Bicarbonate. The effect of Potassium Bicarbonate (2% or 4%) on rat urinary bladder epithelium was studied without prior exposure to a bladder tumor initiator.[36] In 4 studies, ranging in duration from 4 to 130 wk, equimolar amounts of potassium were administered in the diet to male and female weanling, SPF-bred Wistar rats (Cpb:WU; Wistar random) (85 rats/sex/group) as Potassium Bicarbonate. Control rats were fed a cereal-based open formula diet. The feeding of Potassium Bicarbonate (2% and 4% concentrations) resulted in simple epithelial hyperplasia and, after prolonged administration, in papillary/nodular hyperplasia, papillomas, and transitional cell carcinomas of the urinary bladder. The incidence of hyperplastic and neoplastic bladder lesions tended to be higher in rats fed 4% Potassium Bicarbonate than in those fed 2% Potassium Bicarbonate, suggesting a dose-response relationship. Based on these results, the authors concluded that Potassium Bicarbonate is capable of inducing urinary bladder cancer in rats without prior application of an initiator.

Groups of 10 weanling male SPF-bred albino rats (Cpb: WU; Wistar random) were fed a basal diet or diet supplemented with Potassium Bicarbonate (2.5% in the diet) for up to 13 wk.[37] A group of 10 rats was also fed 6% monosodium glutamate (MSG) in the diet. The rats that received 6% MSG in the diet showed an increased incidence and degree of focal and diffuse hyperplasia of the bladder epithelium. The group that received Potassium Bicarbonate in the diet also had epithelial hyperplasia in the urinary bladder. Hyperplasia of the epithelium lining the renal pelvis or papilla was not observed in these animals.

Another 13-week study was performed using groups of 10 weanling male SPF-bred albino rats (Cpb: WU; Wistar random) to compare the effects of 5% Potassium Bicarbonate in a stock diet and in a purified diet.[37] Unsupplemented stock and purified diets served as controls. The rats were gradually accustomed to the high level of Potassium Bicarbonate (5%) by feeding 1% in the diet during week 1, 2% in the diet during week 2, 3% in the diet during week 3, 4% in the diet during weeks 4 and 5, and 5% in the diet from week 6 on. The microscopic examinations of the urinary bladder, ureters, kidneys, liver, testes, thyroid with parathyroids, adrenals, and bone revealed changes considered to be related to treatment only in the bladder epithelium. These changes comprised various forms and degrees of epithelial hyperplasia and very small intra-epithelial cysts. An increased incidence and severity of hyperplasia occurred in each of the two groups that received Potassium Bicarbonate. Generally, the hyperplastic changes were diffuse, and their degree varied from minimal to moderate. More severe hyperplasia (papillomatous) was present in one rat fed Potassium Bicarbonate in the stock diet. Both the incidence and the degree of the epithelial changes indicated a more marked effect of Potassium Bicarbonate in the stock diet than in the purified diet.

In a 30-mo carcinogenicity study, groups of 50 SPF-bred weanling Wistar rats (CpB:WU;Wistar random) per sex were fed a natural ingredient diet (controls) or diet supplemented with 2% or 4% Potassium Bicarbonate.[34] There were no treatment-related mortalities. At necropsy, macroscopic examination did not reveal any significant differences among test and control groups, except for macroscopic lesions in the urinary bladder of some rats. Most histopathological changes observed were considered equally distributed among the treatment groups and the controls and represented normal background pathology for rats of this strain and age.

Dose-related increases in the incidence of zona glomerulosa hypertrophy (classified as a non-neoplastic histopathological change) occurred in all treatment groups (both sexes) and was statistically significant ($P < .01$) when compared to the control. At week 13, oncocytic tubules were noted in males and females fed 2 or 4% Potassium Bicarbonate; after 30 mo, the incidence of this lesion was much higher in the treated rats when compared to the background incidence in controls. No progression to oncocytomas was noted. The incidences of simple epithelial hyperplasia and of papillary/nodular hyperplasia of the urinary bladder were increased in the 2% and 4% Potassium Bicarbonate groups. Urothelial hyperplasia (classified as a non-neoplastic histopathological change) and papillary/nodular hyperplasia of the urinary bladder were statistically significant ($P < .01$), compared to the control. The incidence of (multiple) transitional cell papilloma (benign) in the urinary bladder was 2 (in males)

and 6 (in females; $P < .05$) for animals dosed with 4% Potassium Bicarbonate. The incidence of transitional cell carcinoma (malignant) in the urinary bladder was 1 (in males) and 3 (in females) dosed with 4% Potassium Bicarbonate. These changes indicate an association between prolonged treatment with Potassium Bicarbonate and urinary bladder cancer. Except for the preneoplastic and neoplastic lesions in the urinary bladder, there were no treatment-related changes in any specific tumor type among the groups. In females, relatively high incidences of adenocarcinomas were found in the uterus with 4% Potassium Bicarbonate, but because these changes were not accompanied by preneoplastic alterations in this 30-mo study or in the 18-mo chronic oral study (in Chronic Oral Toxicity section) and because their incidences were within the range of historical control data, they were not deemed treatment-related.

Additionally, the total number of rats with tumors and the total incidence of tumors were not affected by treatment. Although the number of Potassium Bicarbonate-fed males with malignant tumors reached the level of statistical significance, the difference compared to the controls was not statistically significant when the number of urinary bladder lesions was excluded from the evaluation. In summary, apart from the effects on the urinary bladder, treatment with Potassium Bicarbonate did not affect the type, incidence, or multiplicity of tumors, or the time of tumor appearance or the ratio of benign-to-malignant tumors.[34]

Co-Carcinogenicity

Sodium Bicarbonate

The co-carcinogenicity of sodium bicarbonate in the presence of *o*-phenylphenol was evaluated using groups of Fischer 344 male rats.[30] The 6 dietary groups (31 rats/group) in this study were: Group 1 (2% sodium *p*-phenylphenol [OPP-Na] in diet), Group 2 (1.25% OPP + 0.64% sodium bicarbonate in diet), Group 3 (1.25% OPP + 0.32% sodium bicarbonate in diet), Group 4 (1.25% OPP + 0.16% sodium bicarbonate in diet), Group 5 (1.25% OPP or 0.64% sodium bicarbonate in diet), and Group 6 (1.25% OPP or 0.64% sodium bicarbonate in diet). The control group (fed plain diet) consisted of 30 rats. The groups were fed continuously for 104 wk. In week 104, the % survival was 84% (26 of 31 rats) for animals exposed to sodium bicarbonate and 73% (22 of 30 rats) in the control group. When compared to the control, the final body weight was statistically significantly lower in all dietary groups. The relative weight (organ/body weight %) of the kidneys and liver was statistically significantly increased when compared to the control. Also, when compared to the control group, animals fed sodium bicarbonate in the diet did not have a statistically significant increase in the number of tumors. The first bladder tumor was observed in the rat that died in week 49. The number of rats that survived for 104 wk is unknown. Sodium bicarbonate alone in the diet did not have a carcinogenic effect on the urinary bladder of rats. Papillary or nodular hyperplasia and papilloma incidence did not differ between test animals and the control group.

Tumor Promotion

Sodium Bicarbonate

The following data on sodium bicarbonate (surrogate compound) are included in a REACH dossier on Ammonium Carbonate.[29] The tumor promotion potential of sodium bicarbonate in male 344 rats dosed with the carcinogen *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) was evaluated in a 32-wk study. Ten groups of 20 rats received drinking water with 0.05% BBN and 2 groups of 10 rats (control groups) received drinking water without BBN for the first 4 wk of the study. At 3 d after the cessation of BBN treatment, 4 groups received a powdered basal diet containing 0.375%, 0.75%, 1.5%, and 3% sodium bicarbonate, respectively; the control group received basal diet only. The remaining 5 groups consisted of 4 groups that received powdered basal diet containing the 4 concentrations of sodium bicarbonate, respectively, + 5% ascorbic acid and the control group that received basal diet + 5% ascorbic acid for 32 wk. The 2 groups of 10 rats (controls) that received drinking water without BBN were maintained on powdered basal diet containing 3% sodium bicarbonate or 5% ascorbic acid + 3% sodium bicarbonate for 32 wk. The total observation period was 36 wk and 3 d. In a second experiment, the rats were randomly divided into 4 groups of 5 rats and 4 groups received powdered basal diet with the following components, respectively: 3% sodium bicarbonate, 5% ascorbic acid, 3% sodium bicarbonate + 5% ascorbic acid. The fifth group (control) received basal diet with no added chemicals.

In 3 of the 10 groups dosed with BBN, the urinary bladder had multiple large tumors. Papillary or nodular hyperplasia and papillomas were observed. Urinary pH and sodium concentrations were increased in rats fed sodium bicarbonate only if they had been pretreated with BBN. Similar results were not reported for animals fed sodium bicarbonate only. The results of this study confirmed that the dose-dependent increase in both urinary pH and sodium concentration and the dose-dependent promotion of urinary bladder carcinogenesis were parallel effects of sodium bicarbonate. Study results also indicated that ascorbic acid administered orally acted as an amplifier (a co-promoter), though this vitamin had no promotion potential.[29]

Other Relevant Studies

Nephrotoxicity

The possible toxic effects of a Potassium Carbonate emulsion (pH not stated) on some biomarkers of tissue damage was investigated using groups of 4 California rabbits.[43] The rabbits

Table 4. Skin Irritation/Sensitization Studies.

Test Substance	In Vitro Assays/Animals Tested	Test Protocol	Results
Skin Irritation Studies			
In Vitro Studies			
Magnesium Carbonate	Reconstituted human epidermis model.	OECD Guideline 431. Solid material (20 mg) applied topically to model, followed by addition of 0.9% w/v sodium chloride solution for wetting of test material. Duplicate tissues treated for exposure periods of 3, 60, and 240 minutes. Negative control (0.9% w/v sodium chloride solution) and positive control (glacial acetic acid).	Non-corrosive to the skin.[23]
Ammonium Bicarbonate	Reconstructed 3-dimensional human epidermis model	OECD Guideline 431.25 μl (concentration not stated) applied to epidermal tissue samples for up to 1 h. Colorimetric test to determine tissue destruction by measuring metabolic activity of tissue. Reduction of mitochondrial dehydrogenase activity measured by reduced formazan production after incubation with tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)].	Ammonium Bicarbonate unable to reduce MTT directly in this assay. Mean viability of treated tissues was 105% after 3- minute exposure and 36% after 1-h exposure.[25]
Ammonium Bicarbonate	Reconstructed 3-dimensional human epidermis model	Skin corrosion test (OECD Guideline 431). Epidermal tissue samples incubated with test substance for 1 h, followed by 42-h post-incubation period.	Mean viability of treated tissues was 71% after 1-h exposure +42-day post-incubation period. Test substance classified as non-irritating.[25]
Ammonium Carbonate	3-Dimensional human epidermis model	OECD Guideline 439. Test substance (50 μl, concentration not stated) applied to disks of tissue samples, followed by 15-minute incubation period and 42-h post-incubation period. Viability of each disk assessed by incubating tissues for 3 h with MTT solution.	Mean tissue viability was 115%. Test substance classified as non-irritating.[29]
Calcium Carbonate	3-Dimensional human epidermis model	In vitro test method for assessing dermal corrosivity potential. Methodology based on ability of corrosive chemical to pass through a biobarrier and elicit color change in underlying liquid Chemical Detection System (CDS). Biobarrier composed of a hydrated collagen matrix in supporting filter membrane. CDS composed of water and pH indicator dyes.	Test substance classified as non-corrosive.[44]
Animal Studies			
Calcium Carbonate	Rabbits (number and strain not stated)	Undiluted test substance applied to intact skin. Evaluation for corrosion within 3 minutes and at 1 h or 4 h post-application.	Test substance classified as non-corrosive.[44]

(continued)

Table 4. (continued)

Test Substance	In Vitro Assays/Animals Tested	Test Protocol	Results
Potassium Bicarbonate	6 New Zealand white rabbits	Test substance (500 mg in saline) applied to dorsal area of trunk (abraded or intact skin). Site covered with 2.5 cm^2 occlusive patch for 24 h	At 24 h and 72 h post-application, no signs of erythema or edema at intact skin sites. However, at both times, very slight erythema (score= 1), with slightly white discolorations, at abraded sites of all animals. No evidence of edema. Primary irritation index (abraded and intact skin; maximum possible index= 8)= 0.5. Test substance classified as mild skin irritant.[27]
Potash Hydrate (composition not stated; surrogate for Potassium Carbonate)	6 New Zealand white rabbits	Test substance (500 mg moistened with saline) applied, under 2.5 cm x 2.5 cm occlusive dressing, to abraded and intact dorsal skin for 24 h.	Skin irritation not observed at intact sites at 24 h or 72 h post-application. For abraded sites, erythema score of 4 and edema score of 2 in all rabbits at 24 h. At 72 h, erythema score of 4 (abraded skin) in all rabbits. Primary irritation index (abraded and intact skin scores)= 2.5. Test substance classified as moderate skin irritant.[28]
Ammonium Carbamate (component of Ammonium Carbonate)	2 Vienna white rabbits	Test substance (40% or 80% aqueous; 1 ml) applied, under occlusive patch, to skin for 1, 5, or 15 minutes. Application followed by 24-h observation period.	Neither local effects nor clinical symptoms observed. Test substance classified as non-irritant at both concentrations.[30]
Ammonium Carbamate (component of Ammonium Carbonate). Contained 43.6% ammonium and 56.3% carbon dioxide.	10 rats (5 males, 5 females) of the Crl:CD(SD) strain	Test substance (in water; dose= 5000 mg/kg body weight) applied, under semi-occlusive patch, to the back for 24 h.	Erythema (very slight to severe), edema (very slight to moderate), eschar, necrosis, exfoliation, scabbing, and desquamation were reported. Number of animals with reactions not stated.[30]
Sensitization Studies			
In Vivo Studies			
Calcium Carbonate (solid nanomaterial, white powder)	Three groups of 4 mice of the CBA/Ca (CBA/caOlaHsd) strain	Local lymph node assay (OECD Guideline 429). Three groups exposed to concentrations of 5%, 10%, and 25% w/w, respectively. Each concentration (in formamide) applied (25 μl) to dorsal surface of ear for 3 consecutive days. Hexyl cinnamic aldehyde served as positive control.	Calcium Carbonate was a non-sensitizer. Positive control was a sensitizer; stimulation index, expressed as the mean radioactive (^{3}HTdR) incorporation into lymph node cells for the treatment group divided by the mean radioactive incorporation of the control groups, was 5.6.[26]
Ammonium Acetate (surrogate for Ammonium Carbonate)	3 groups of 4 CBA female mice	Local lymph node assay (OECD Guideline 429). Same as above procedure except for test concentrations of 10% w/v, 25% w/v, and 50% w/v. Tested as 25 μl in acetone/olive oil [4:1 v/v] mixture	No signs of irritation at application site in any treatment group. Stimulation index values were 1.6 (for 10%), 1.7 (for 25%) and 1.3 (for 50%). Stimulation index for positive control was 10.7. Non-sensitizer[29]
Ammonium Carbamate (component of Ammonium Carbonate)	3 groups of 5 mice of the CBA/J inbred SPF strain	Local lymph node assay (OECD Guideline 429). Same test concentrations as in above procedure. Tested as 25 μl in propylene glycol.	Test substance was a non-sensitizer. Stimulation index values were 1.1 (for 10% ammonium carbamate), 1.2 (for 25% ammonium carbamate), and 0.6 (for 50% ammonium carbamate).[30]

(continued)

Table 4. (continued)

Test Substance	In Vitro Assays/Animals Tested	Test Protocol	Results
Animal Studies			
Potassium Bicarbonate	Groups of Hartley guinea pigs of the (Crl:HA)BR strain (10 test Guinea pigs, 10 negative controls, and 4 positive controls)	Buehler test procedure. Induction: test substance (0.2 g in deionized water) applied, under an occlusive patch (Hill Top Chamber®, 25-mm diameter), for 6 h to the anterior left flank once per week for 3 weeks. Challenge: Test substance (0.2 g in deionized water) applied to anterior right flank. Positive controls treated with 0.3% w/v 2,4-dinitrochlorobenzene.	Test substance was non-sensitizer.[27]
Potassium Carbonate	Groups of guinea pigs of unspecified strain (10 test guinea pigs, 10 negative controls, and 5 positive controls)	Buehler test procedure. Test substance (moistened with distilled water) applied for 24 h, under occlusive patch, to the skin at a concentration of 95% w/w during 3-wk induction period and challenge phase. Dinitrochlorobenzene served as positive control.	Test substance was non-irritant and non-sensitizer.[28]
Ammonium Chloride (surrogate for Ammonium Bicarbonate)	20 Pirbright-Hartley guinea pigs	Maximization test. Induction phase consisted of single intracutaneous induction exposure to 5% ammonium chloride and a single 48-h epicutaneous induction exposure to 25% ammonium chloride. Intradermal injection occurred on day 1 and epicutaneous induction occurred on day 9. For epicutaneous induction, a 2 × 4 cm occlusive patch containing 0.5 ml of the test substance was applied to the area of the intradermal injection site. On day 22, animals received a 24-h challenge application (occlusive patches) of 10% ammonium chloride.	Two Guinea pigs had + reactions (barely perceptible erythema) with very slight to slight edema. 10% of the animals tested had a positive reaction after being challenged with ammonium chloride. Results were interpreted as the absence of skin sensitization potential[25]

received Potassium Carbonate emulsion orally, via drinking, at doses of 50 mg/l and 100 mg/l for 14 consecutive days. The control group received physiological saline. At the higher concentration (100 mg/l), the emulsion significantly increased uric acid, creatinine, and urea in the urine by 126.3%, 48.6%, and 458.8%, respectively, compared to the control ($P < .05$). Oral administration of the emulsion at a concentration of 50 mg/l caused a statistically significant increase in urea, creatinine, and uric acid by 253.8%, 38.6%, and 88.8%, respectively, compared to the control. Also, Potassium Carbonate emulsion statistically significantly increased serum blood urea nitrogen (BUN) at concentrations of 50 mg/l and 100 mg/l. The results of this study suggested that oral exposure to excessive amounts of Potassium Carbonate emulsion repeatedly over an extended period could precipitate kidney damage.

Dermal Irritation and Sensitization Studies

Skin Irritation and Sensitization

Skin irritation and sensitization studies on carbonate salts are summarized in Table 4.[23,25-32,44] The results for in vitro skin irritation studies were negative, however, a surrogate chemical for Potassium Carbonate was classified as a skin irritant in rabbits and ammonium carbamate (component of Ammonium Carbonate) induced skin irritation in an acute dermal toxicity study involving rats. Additionally, negative results were reported in local lymph node assays evaluating sensitization potential, and the same was true for animal sensitization tests (Buehler and maximization tests included) involving mice or guinea pigs.

Ocular Irritation Studies

In Vitro

Magnesium Carbonate. The ocular irritation potential of Magnesium Carbonate (concentration not stated) was evaluated in the in vitro bovine corneal opacity and permeability test (BCOP).[45] In the BCOP test method, changes in corneal opacity caused by chemical damage are determined by measuring decreases in light transmission through the cornea. Changes in permeability of the cornea resulting from chemical damage are determined by measuring increases in the quantity

of sodium fluorescein dye that passes through all corneal cell layers. Both measurements are used to calculate an in vitro irritancy score (IVIS), which is used to predict the in vivo ocular irritation/corrosion potential of a test substance. The following scores are considered positive: corneal opacity (CO) or iris (IR) score ≥1 or conjunctival chemosis (CC) or conjunctival redness (CR) ≥2. There was no evidence of CC or CR or lesions of the iris. A CO score of 1 was reported, and the reaction cleared by day 3. Therefore, Magnesium Carbonate caused only corneal opacity in this test.

Ammonium Bicarbonate

The Hen's Egg Chorioallantoic Membrane Test (HET-CAM) was used to evaluate the ocular irritation potential of Ammonium Bicarbonate.[25] Undiluted Ammonium Bicarbonate (25 µl) or 10% aqueous Ammonium Bicarbonate (0.3 ml) was applied topically to the chorioallantoic membrane of fertilized and incubated hen eggs (3 eggs per test concentration. There was no evidence of irritation at the 10% concentration. Undiluted Ammonium Bicarbonate caused moderate intravascular coagulation in all eggs within 50 sec. Hemorrhagia was not noted during the observation period. It was concluded that, under the conditions of this test, Ammonium Bicarbonate did not produce changes that were indicative of serious eye damage.

Animal

Magnesium Carbonate. In a study involving 2 New Zealand white rabbits, the ocular irritation potential of Magnesium Carbonate (10% w/v aqueous, pH 9.9) was evaluated.[23] Magnesium Carbonate (10 ml) was instilled into the conjunctival sac of the right eye. The left eye served as the untreated control. Eyes were not rinsed after instillation of the test substance. Moderate conjunctival irritation was observed at 1 h post-instillation, and minimal conjunctival irritation was observed at 24 h and 48 h post-instillation. Reactions had cleared by 72 h post-instillation. Reactions were not observed in the cornea or iris. Magnesium Carbonate was classified as non-irritating to the eyes of rabbits.

Ammonium Carbonate

The ocular irritation potential of Ammonium Carbonate was evaluated using 3 New Zealand white rabbits.[29] Ammonium Carbonate (0.1 ml; ~66 mg) was instilled into one eye. The following reactions were observed: slight corneal opacity, moderate conjunctival redness, slight to marked conjunctival chemosis, and slight to severe discharge. Additionally, discharge of blood, scleral vessels injected in a circumscribed area, or circular and marginal vascularization of the cornea in a circumscribed area were observed. The ocular reactions were reversible in 2 rabbits within 7 d and in 1 rabbit within 14 d after instillation. Ammonium Carbonate was classified as non-irritating.

Calcium Carbonate

The ocular irritation potential of Calcium Carbonate was studied using 3 male New Zealand white rabbits.[26] The test substance (0.1 ml) was instilled into the right eye, and the observation period was up to 72 h. Left eyes served as controls. Ocular damage/irritation was evaluated according to the Draize scale. Corneal effects were not observed during the study. Iridial inflammation and minimal conjunctival irritation were observed in all treated eyes at 1 h post-instillation. Conjunctival irritation was also observed at 24 h and 48 h post-instillation. All reactions had cleared by 72 h post-instillation. Calcium Carbonate was classified as non-irritating to the eyes of rabbits.

Potassium Bicarbonate

Undiluted Potassium Bicarbonate (0.1 ml) was instilled into the eyes of 6 male New Zealand white rabbits.[27] The eyes were not rinsed and untreated eyes served as controls. Instillation of the test substance was followed by a 7-d observation period. Mild or moderate conjunctival redness and discharge and moderate or severe chemosis were observed in all animals at 1 h post instillation. At day 4, chemosis and discharge were fully reversible in all animals. The test substance did not induce substantial corneal opacity or iritis. The study was terminated before all ocular reactions were completely reversed. However, the authors noted observations relating to the healing process indicated that the complete reversibility of ocular reactions was likely. Therefore, Potassium Bicarbonate was classified as non-irritating to the eyes of rabbits.

Sodium Carbonate Monohydrate

Data on sodium carbonate monohydrate were used to evaluate the ocular irritation potential of Potassium Carbonate in 9 New Zealand white rabbits.[28] The test substance (0.1 ml; concentration not stated) was instilled into the conjunctival sac, after which the eye was either rinsed (3 rabbits) or not rinsed (6 rabbits). Untreated eyes served as controls. Reactions were scored according to the Draize scale for up to 14 d post-instillation. Conjunctival redness was observed in all 6 rabbits that were not subjected to ocular rinsing, and in 1 rabbit after ocular rinsing. Conjunctival chemosis was observed in all 6 rabbits (no ocular rinsing) and in 2 rabbits after ocular rinsing. Corneal opacity, ulceration, and pannus were also observed in rinsed eyes. Necrosis/ulceration, alopecia, and bleeding were observed in eyes that were not rinsed. Signs of ocular irritation persisted to the end of the study in rabbits (unrinsed eyes) and in one rabbit (no ocular rinsing). It was concluded that sodium carbonate monohydrate was irritating to the eye.

Clinical Studies

Case Reports

Calcium Carbonate. A female patient (history of gastritis) at 36 wk of gestation had ingested 5 glasses of milk and

approximately 30 tablets of an antacid containing 500 mg of Calcium Carbonate daily for 2 wk.[26] A Calcium Carbonate intake of >5.5 g/d for 14 d during pregnancy (later stages) did not cause any observable teratogenic effects.

Potassium Carbonate

A male crystal factory worker with a 1-mo history of eczema on the hands, arms, and legs was patch-tested with a 1% aqueous solution of Potassium Carbonate.[28] The patch test procedure was not stated. Patch test results were negative for skin sensitization.

Summary

The carbonate salts have the following functions in cosmetic products: absorbents, bulking agents, opacifying agents, pH adjusters, buffering agents, abrasives, and oral care agents.

Collectively, information supplied to FDA by industry as part of the VCRP and a survey of ingredient use concentrations conducted by the Council indicate that the following carbonate salts are being used in cosmetic products: Magnesium Carbonate, Ammonium Bicarbonate, Ammonium Carbonate, Calcium Carbonate, and Potassium Carbonate. The highest use frequency is reported for Magnesium Carbonate (317 uses). The Council survey data also indicate that the carbonate salts are being used in cosmetics at maximum ingredient use concentrations up to 93.4% (i.e., Ammonium Bicarbonate in rinse-off products [hair bleaches]). The highest maximum concentration of use in leave-on products is being reported for Calcium Carbonate (concentrations up to 35% in eyebrow pencils).

Calcium Carbonate (0.40 mCi of calcium [^{14}C]carbonate pellet) was implanted intraperitoneally into a male rat.[24]

Approximately 72% of the radiolabeled carbonate was excreted as respiratory carbon dioxide between 2 h and 142 h after implantation.

After magnesium sulfate was fed (in the diet) to rabbits, mean urinary excretion of radioactivity ranged between 10% and 12.5%, and the cecum and its contents were found to contain 78% of the ingested dose. After [^{11}C]sodium bicarbonate was injected intraperitoneally into rats, the urine contained 1.3% of the radioactivity and >50% of the radioactivity occurred as respiratory [^{11}C]carbon dioxide.

Following ingestion, Potassium Carbonate rapidly dissociates in the gastric juice to yield carbonate ions and potassium ions. Similarly, after ingestion, Potassium Bicarbonate rapidly dissociates in gastric juice to yield carbonate ions (bicarbonate and carbonate) and potassium ions. The absorbability of calcium from Calcium Carbonate and calcium citrate salts was compared in a study in which 37 men and women ingested 300 mg (low load) and 1000 mg (high load) calcium loads as part of a light breakfast meal. Relative absorption was estimated from the difference in urinary calcium following breakfast on the 2 d and true fractional absorption from the appearance of [^{45}Ca] in the serum. Mean tracer absorption for both salts combined was 36% at the 300 mg load and 28.4% at the 1000 mg load.

In an acute dermal toxicity study on a tradename material (Potassium Carbonate containing pesticide), the LD_{50} in rabbits was >2000 mg/kg body weight. The same results (LD_{50} > 2000 mg/kg) were reported in acute dermal toxicity studies on Ammonium Carbonate, Calcium Carbonate, Potassium Bicarbonate, and Ammonium Sulfate.

An acute oral LD_{50} of ~2000 mg/kg was reported for Ammonium Bicarbonate, Ammonium Carbonate and Potassium Carbonate in studies involving rats. The acute oral LD_{50} for Calcium Carbonate, and Potassium Bicarbonate was >2000 mg/kg.

In an acute inhalation toxicity study on ammonia (1-h exposure) involving mice, the lungs of animals that died were diffusely hemorrhagic (mild to moderate pneumonitis also observed) and liver damage was observed at each concentration of exposure (3440 ppm, 4220 ppm, and 4860 ppm). It was noted that the liver lesions may have resulted from the compromised nutritional state of the mice. An acute inhalation LC_{50} value (1-h exposure) of 6.8 mg/l air for Ammonium Bicarbonate was deduced based on the results of this study.

An acute inhalation LC_{50} of >3 mg/l of air was reported for rats exposed to aerosolized Calcium Carbonate. In acute inhalation toxicity studies on Potassium Bicarbonate and sodium bicarbonate, an LC_{50} value of >5 mg/l of air was reported. In an acute inhalation toxicity study on a tradename material (Potassium Carbonate containing pesticide), a mean LC_{50} of >5 mg/l air (rats) was reported.

Mean acute i.v. toxicity values of 3.10 and 1.02 mM/kg were reported for Ammonium Bicarbonate and Ammonium Carbonate, respectively, in studies involving albino rats.

A NOAEL of 1000 mg/kg body weight/day was reported in a study in which male and female rats received oral doses of magnesium chloride hexahydrate for 14 d pre-mating and 14 d of mating. The same results were reported for female rats dosed orally during gestation and up to PND 3 and for male rats dosed orally for 28 to 29 d. Based on the results of the preceding 2 experiments, it was determined that the equivalent NOAEL for Magnesium Carbonate was 4128 mg/kg body weight/d. Rats fed Calcium Carbonate at 300 mg/kg for 3 d had no indication of toxicity, and the same was true for Calcium Carbonate (nano form) at oral doses up to 1000 mg/kg body weight/day for 14 d. There were no test substance-related changes in rats fed doses up to 4228.5 mg/kg body weight/d ammonium chloride for 28 d.

In repeated dose oral toxicity studies (4-wk, 13-wk, 18-mo, and 30-mo studies) of Potassium Bicarbonate (2% or 4% in diet), most of the histopathological changes observed were considered equally distributed among treatment groups, and represented normal background pathology for SPF-bred Wistar rats. Neither gross nor microscopic changes of the urinary bladder were observed in rats fed ammonium chloride (in the diet) in a short-term (70 d) oral feeding study. The NOEL was <684 mg/kg body weight/d.

Inhalation exposure to an aerosolized Potassium Carbonate-based scrubbing solution for 21 d did not result in any persistent systemic toxicity or neurotoxicity in either male or female rats.

The results of a study in which rabbits were dosed orally (via drinking) with Potassium Carbonate emulsion (50 mg/l and 100 mg/l) for 14 consecutive days suggested that Potassium Carbonate emulsion exposure could precipitate kidney damage.

The reproductive and developmental toxicity of magnesium chloride hexahydrate was evaluated in male and female rats that received oral doses up to 1000 mg/kg body weight/day for 14 d pre-mating and 14 d of mating. Additionally, female rats received the same doses during gestation and up to PND 3 and male rats were dosed similarly for 28 to 29 d. The NOAEL for the reproductive/developmental toxicity of magnesium chloride hexahydrate was 1000 mg/kg body weight/d; the equivalent NOAEL for Magnesium Carbonate was determined to be 414 mg/kg body weight/d. In another study, pregnant female rats were dosed orally with magnesium chloride hexahydrate at doses (in distilled water) up to 800 mg/kg body weight/d on gestation days 6 to 15. Visceral malformations were observed in 4 to 6 fetuses from each dose group, but without intergroup differences. The NOAEL for magnesium chloride hexahydrate was estimated to be >800 mg/kg body weight/d; the equivalent NOAEL for Magnesium Carbonate was determined to be >331 mg/kg body weight/d.

Female Swiss mice were bred after feeding with a diet supplemented with 0.5%, 1%, or 2% Calcium Carbonate. Calcium Carbonate (2%) in the diet caused hypertrophy of the heart and decreased thymus weight in weanling mice. In a reproductive toxicity study, rats were fed oral doses of Calcium Carbonate (nano form) up to 1000 mg/kg body weight/d for 48 consecutive days. The maximum dose administered was the NOEL in this study. In a 62-d developmental toxicity study, rats were fed Calcium Carbonate at concentrations up to 1.25% in the diet, and the NOAEL for teratogenicity was determined to be >1.25% in the diet.

There was no evidence of embryotoxicity or teratogenicity in the offspring of rats dosed orally with sodium bicarbonate (up to 340 mg/kg body weight) on gestation days 6 through 15. In another study on sodium bicarbonate, there were no test substance-related abnormalities in the offspring of rats that received sodium bicarbonate at concentrations of 0.5% and 2% (in drinking water) on gestation days 15 through 20. The number of abnormalities observed in soft or skeletal tissues in groups of mice that received oral doses of sodium bicarbonate up to 580 mg/kg on gestation days 6 through 15 did not differ from the number occurring spontaneously in the sham-treated controls.

In teratogenicity studies involving rats and mice, NOELs of 290 mg/kg and 180 mg/kg (highest dose in each study), respectively, were reported for Potassium Carbonate. The results of an inhalation developmental toxicity study on a Potassium Carbonate scrubbing solution (up to 3 mg/l) were negative.

A female patient at 36 wk of gestation ingested 5 glasses of milk and approximately 30 tablets of an antacid containing 500 mg of Calcium Carbonate daily for 2 wk. Teratogenic effects were not observed.

In the chromosome aberrations assay, Calcium Carbonate was non-clastogenic to human lymphocytes and magnesium chloride and ammonium sulfate were non-genotoxic to Chinese hamster lung fibroblast (V79) cell cultures. Ames test results for ammonium carbamate, Potassium Bicarbonate, and Potassium Carbonate were negative for genotoxicity. Results were negative for potassium chloride in the L5178Y mouse lymphoma cell mutagenesis assay. In the in vivo micronucleus test, ammonium chloride was not genotoxic.

Additionally, anti-genotoxic effects of Magnesium Carbonate have been reported in in vitro tests involving *Saccharomyces cerevisiae* strain D4, Balb 3T3 fibroblast cells, or Chinese hamster ovary cells, but not in the Ames test using *S. typhimurium* strain 102.

In a co-carcinogenicity study in which rats were fed sodium bicarbonate (up to 0.64% in diet) + OPP (1.25% in the diet) continuously for 104 wk, there was no statistically significant increase in the number of bladder tumors when compared to the control group. Also, sodium bicarbonate alone did not have a carcinogenic effect on the urinary bladder.

Bladder cancer has been associated with rats fed Potassium Bicarbonate in the diet (2% or 4%) for up to 130 wk. The results of a 32-wk tumor promotion study involving rats dosed with sodium bicarbonate (up to 3% in the diet) and 0.05% BBN confirmed that the dose-dependent increase in both urinary pH and sodium concentration and the dose-dependent promotion of urinary bladder carcinogenesis were parallel effects of sodium bicarbonate.

Undiluted Magnesium Carbonate, Ammonium Bicarbonate, and Ammonium Carbonate were non-irritating/non-corrosive to the reconstituted human epidermis model in vitro. Also, using an in vitro test for assessing dermal corrosivity potential (Corrositex®), undiluted Calcium Carbonate was not found to be a corrosive agent.

Undiluted Calcium Carbonate was not a corrosive agent when applied to the skin of rabbits. In the local lymph node assay, Calcium Carbonate (solid nanomaterial) was applied to the skin of mice at concentrations up to 25%, and the results of this assay were negative.

Potash hydrate (surrogate chemical for Potassium Carbonate; 500 mg in saline) was moderately irritating to the skin of 6 rabbits and Potassium Bicarbonate (500 mg in saline) was classified as mildly irritating to the skin of 6 rabbits. Reactions were observed at abraded sites, but not intact sites. Potassium Bicarbonate (0.2 g in deionized water) was classified as a non-sensitizer in the Buehler test (10 guinea pigs). Reactions were not observed during induction or the challenge phase. In a repeated insult patch test, a Potassium Carbonate tradename material (tested at 95% w/w) also was not a skin sensitizer in the Buehler test (10 guinea pigs). In the local lymph node assay, ammonium acetate was applied to the skin of mice at

concentrations up to 50%, and the results of this assay were negative (non-sensitizer). Ammonium carbamate also produced negative results in the local lymph node assay (non-sensitizer) when applied to the skin of mice at concentrations up to 50%.

Magnesium Carbonate caused only corneal opacity in the in vitro bovine corneal opacity and permeability test. In the HET-CAM test, it was concluded that Ammonium Bicarbonate (undiluted or 10% aqueous) did not produce changes that were indicative of serious eye damage. Undiluted Ammonium Bicarbonate, but not the 10% concentration, caused intravascular coagulation in all eggs.

Magnesium Carbonate (10% aqueous) was classified as non-irritating to the eyes of 2 rabbits. However, transient ocular irritation was observed. Undiluted Ammonium Carbonate (3 rabbits tested), Calcium Carbonate (3 rabbits tested), and Potassium Bicarbonate (6 rabbits tested) were classified as non-irritating to the eyes of rabbits. The ocular reactions observed were reversible. In a study involving 9 rabbits, undiluted sodium carbonate monohydrate was classified as an ocular irritant.

Discussion

The Panel noted that this safety assessment contains toxicity data on ammonium carbamate because the cosmetic ingredient Ammonium Carbonate is made up of Ammonium Bicarbonate and ammonium carbamate.

The Panel expressed concern about the potential for skin and ocular irritation from exposures to carbonate salts. For example, animal studies reported that Potassium Carbonate and sodium carbonate monohydrate (a closely related chemical that is not a cosmetic ingredient) were skin and ocular irritants, respectively. Sodium carbonate monohydrate is not used; however, other carbonate salts are being used in eye products, but are not irritants. The Panel noted that the carbonate salts alone would not likely be irritating at concentrations used in cosmetic products, but that these ingredients may contribute to the irritation potential of other ingredients in cosmetic formulations. Thus, the Panel determined that cosmetic products containing carbonate salts should be formulated to be non-irritating.

The Panel noted studies that reported renal toxicity and neoplastic lesions of the urinary bladder in animals fed Potassium Bicarbonate. However, the Panel concluded that the effects reported in these studies are attributable to irritation resulting from the ingredient in the urine after repeated daily exposure to high dietary concentrations of Potassium Bicarbonate over an extended period. The Panel agreed that the dietary exposures tested in these studies do not reflect the much lower exposures that can reasonably be expected from the use of carbonate salts in cosmetic products.

Concern about heavy metals that may be present in carbonate salts 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 hair sprays and face powders. They considered pertinent data indicating that incidental inhalation exposures to these ingredients in such cosmetic products would not cause adverse health effects, specifically, short-term (repeated exposures for 21 d) inhalation toxicity data and developmental toxicity data on a scrubbing solution containing 30.8% Potassium Carbonate in studies involving rats. The Panel also 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 the Cosmetic Ingredient Review (CIR) website http://www.cir-safety.org/cir-findings.

Conclusion

The Expert Panel for Cosmetic Ingredient Safety concluded that the following 6 carbonate salts are safe in the present practices of use and concentration, as described in this safety assessment, when formulated to be non-irritating.

Magnesium Carbonate
Ammonium Bicarbonate
Ammonium Carbonate
Calcium Carbonate
Potassium Bicarbonate*
Potassium Carbonate

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

Author's Note

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, DC 20036, USA.

Author Contributions

Johnson, W. contributed to conception and design, contributed to acquisition, analysis, and interpretation, and drafted manuscript; Heldreth, B. contributed to conception and design, contributed to acquisition, analysis, and interpretation, drafted manuscript, and critically revised manuscript; Bergfeld, W., Belsito, D., Hill, R., Klaassen, C., Liebler, D., Marks, J., Shank, R., Slaga, T., and Snyder,

P. contributed to conception and design, contributed to analysis and interpretation, and critically revised manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The articles in this supplement were sponsored by the Cosmetic Ingredient Review.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The articles in this supplement were sponsored by the Cosmetic Ingredient Review. The Cosmetic Ingredient Review is financially supported by the Personal Care Products Council.

References

1. Nikitakis J, Lange B. *International Cosmetic Ingredient Dictionary and Handbook*. 16 ed. Washington, DC:Personal Care Products Council; 2016.
2. Elder RL. Final report on the safety assessment of sodium sesquicarbonate, sodium bicarbonate, and sodium carbonate. *J Am Coll Toxicol*. 1987;6(1):121-138.
3. Andersen FA. Annual review of cosmetic ingredient safety assessments-2004/2005. *Int J Toxicol*. 2006;25(2):1-89.
4. O'Neil MJ. *The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals*. 14th ed. Whitehouse Station, NJ: Merck Research Laboratories; 2006.
5. Industrial Minerals Association of North America. What is calcium carbonate? 2016. Last Updated. http://www.ima-na.org/page/what_is_calcium_carb Date Accessed 4 5, 2016.
6. Federation of American Societies for Experimental Biology. *Evaluation of the Health Aspects of Magnesium Salts as Food Ingredients*. National Technical Information Service (NTIS); 1976:1-32.
7. The United States Pharmacopeial Convention. *Food Chemicals Codex*. 8th ed. Rockville: The United States Pharmacopeial Convention; 2012:58-59.
8. European Commission. *Commission Directive 95/45/EC of 26 July 1995: Laying down specific purity criteria concerning colors fo use in foodstuffs*. 1995;Commission Directive 95/45/EC (E170) on calcium carbonateLast Updated. http://ec.europa.eu/food/fs/sfp/addit_flavor/flav13_en.pdf Date Accessed 27 4, 2016.
9. Food and Drug Administration (FDA). *Information Supplied to FDA by Industry as Part of the VCRP FDA Database*. Washington, DC: FDA; 2016.
10. Personal Care Products Council. Concentration of use by FDA product category - Simple carbonate salts. *Unpublished Data Submitted by the Personal Care Products Council on 4-10-2015*; 2015:1.
11. Rothe H, Fautz R, Gerber E, et al. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104.
12. Bremmer HJ, Prud'homme de Lodder LCH, van Engelen JGM. *Cosmetics Fact Sheet: To Assess the Risks for the Consumer*: 1-77. 2011;Updated version for ConsExpo 4. 20200Report No http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Date Accessed 8 24, 2011.
13. Rothe H. *Special Aspects of Cosmetic Spray Evaluation*. Washington D.C. 2011. Unpublished information presented to the 26 September CIR Expert Panel.
14. Johnsen MA. The influence of particle size. *Spray Technol Mark*. 2004;14(11):24-27. http://www.spraytechnology.com/index.mv?screen=backissues
15. Aylott RI, Byrne GA, Middleton J, Roberts ME. Normal use levels of respirable cosmetic talc: preliminary study. *Int J Cosmet Sci*. 1979;1(3):177-186
16. Russell RS, Merz RD, Sherman WT, Sivertson JN. The determination of respirable particles in talcum powder. *Food Cosmet Toxicol*. 1979;17(2):117-122.
17. CIR Science and Support Committee of the Personal Care Products Council (CIR SSC). 11-3-2015. *Cosmetic Powder Exposure*.
18. Union European. 2015. *Regulation (EC) No. 1223/20098 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Product. Annex IV.* List of Colorants Allowed in Cosmetic ProductsLast Updated. http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02009R1223-20150416&from=EN Date Accessed 12 6, 2016.
19. Food and Drug Administration (FDA). *Title 21. Part 184 - Direct food substances affirmed as generally recognized as safe*. 2021 CFR 184.1135, 184.1137, 184.1191, 184.1425, 184.1613, and 184.1619. 2015.
20. World Health Organization. Ninth Report of the Joint FAO/WHO Expert Committee on Food Additives. *Specifications for the Identity and Purity of Food Additives and Their Toxicological Evaluation: Some Antimicrobials, Antioxidants, Emulsifiers, Satabilizers, Flour-Treatment Agents*. acids, and bases Last Updated 1966 http://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx
21. Jatoi A, Thomas CR Jr. Esophageal cancer and the esophagus: Challenges and potential strategies for selective cytoprotection of the tumor-bearing organ during cancer treatment. *Semin Radiat Oncol*. 2002;12(1):62-67.
22. Food and Drug Administration (FDA). *Inactive Ingredient Search for Approved Drug Products*; 2016. Last Updated http://www.accessdata.fda.gov/scripts/cder/iig/index.Cfm. Date Accessed 4 6, 2016.
23. European Chemicals Agency (ECHA). *Registration, Evaluaton and Authorization of Chemicals (REACH) Dossier. Magnesium Carbonate*. 2016;Last Updated. http://echa.europa.eu/registration-dossier/-/registered-dossier/15234 Date Accessed 20 7, 2016.
24. Federation of American Societies for Experimental Biology. *Evaluation of the Health Aspects of Carbonates and*

Bicarbonates as Food Ingredients. National Technical Information Service (NTIS):1975;1-31.

25. European Chemicals Agency (ECHA). *Registration, Evaluation and Authorization of Chemicals (ECHA) Dossier. Ammonium Bicarbonate*. 2016;Last Updated http://echa.europa.eu/registration-dossier/-/registered-dossier/14575. Date Accessed 20 7, 2016.
26. European Chenmicals Agency (ECHA). *Registration, Evaluation and Authorization of Chemicals (REACH) Dossier. Calcium Carbonate*; 2016. Last Updated http://echa.europa.eu/registration-dossier/-/registered-dossier/16050. Date Accessed 20 7, 2016.
27. European Chemicals Agency (ECHA). *Registration, Evaluation and Authorization of Chemicals (REACH) Dossier. Potassium Bicarbonate*. 2016;Last Updated http://echa.europa.eu/registration-dossier/-/registered-dossier/13190. Date Accessed 26 7, 2016.
28. European Chemicals Agency (ECHA). *Registration, Evaluation and Authorization of Chemicals (REACH) Dossier. Potassium Carbonate*. 2016;Last Updated http://echa.europa.eu/registration-dossier/-/registered-dossier/15221/7/2/2. Date Accessed 12 8, 2016.
29. European Chemicals Agency (ECHA). *Registration, Evaluation and Authorization of Chemicals (REACH) Dossier*. 2016;Ammonium Carbonate:Last Updated http://echa.europa.eu/registration-dossier/-/registered-dossier/11523. Date Accessed 20 7, 2016.
30. European Chemicals Agency (ECHA). *Registraion, Evaluation and Authorization of Chemicals (REACH) Dossier*. 2016;Ammonium Carbamate:Last Updated https://echa.europa.eu/registration-dossier/-/registered-dossier/11611/7/5/2. Date Accessed 4 8, 2016.
31. Environmental Protection Authority. *Ammonium bicarbonate*. Classification data. 2016. Last Updated. http://www.epa.govt.nz/search-databases/Pages/ccid-details.aspx?SubstanceID=3332 Date Accessed 12 7, 2016.
32. Smyth HF, Jr., Carpenter CP, Weil CS, Pozzani UC, Striegel JA, Nycum JS. Range-finding toxicity data: List VII. *American Industrial Hygiene Association Journal*. 1969;30(5):470-476.
33. WilsonDavisMuhrer RPLEME, Davis LE, Muhrer ME, Bloomfield RA. Toxicologic effects of ammonium carbamate and related compounds. *American Journal of Veterinary Research*. 1968;29(4):897-906.
34. Lina BAR, Kuijpers MHM. Toxicity and carcinogenicity of acidogenic or alkalogenic diets in rats; effects of feeding NH 4 Cl, KHCO 3 or KCl. *Food Chem Toxicol*. 2004;42:135-153.
35. BuiClarkNaasUlrich QQCRDJCE, Clark CR, Naas DJ, Ulrich CE, Elangbam CS. A subacute inhalation exposure evaluation of a scrubbing solution used in petroleum refineries. *J Toxicol Environ Health, Part A*. 1998;54:49-62.
36. LinaHollanders BARVMH, Hollanders VMH, Kuijpers MHM. The role of alkalizing and neutral potassium salts in urinary bladder carcinogenesis in rats. *Carcinogenesis*. 1994;15(3):523-527.
37. De GrootFeron APVJ, Immel HR. Induction of hyperplasia in the bladder epithelium of rats by a dietary excess of acid or base: Implications for toxicity/carcinogenicity testing. *Food Chem Toxicol*. 1988;26(5):425-434.
38. Food and Drug Research Laboratories, *Inc. Teratological evaluation of potassium carbonate*. National Technical Information Service (NTIS). 1975. pp.1-38.
39. BuiClarkStump QQCRDG, Nemec ND. Developmental toxicity evaluation of a scrubbing solution used in petroleum refineries. *J Toxicol Environ Health, Part A*. 1998;53:211-222.
40. Bionetics Litton. *Mutagenicity Evaluation of FDA 75-90. Potassium Bicarbonate*. 1977;National Technical Information Service (NTIS). Last Updated https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml.
41. MitchellRudd ADCJ, Caspary WJ. Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Intralaboratory results for sixty-three coded chemicals tested at SRI International. *Environ Mol Mutagen*. 1988;12(13):37-101.
42. Della CroceAmbrosini CC, Cini M, Bronzetti G. Effect of magnesium salts on mitotic gene conversion and point mutation induced by hydrogen peroxide in yeast *Saccharomyces cerevisiae*. *Med.Biol.Environ*. 1996;24(1):51-60.
43. AkintundeOpeolu JKBO, Aina O. Exposure to potassium carbonate emulsion induced nephrotoxicity in experimental animals. *Jordan J Biol Sci*. 2010;3(1):29-32.
44. National Toxicology Program (NTP). *Corrositex®: An in Vitro Test Method for Assessing Dermal Corrosivity Potential of Chemicals. The Results of an Independent Peer Review Evaluation*; 1999. Last Updated Date Accessed 1-27-2016.
45. National Toxicology Program (NTP). *In vivo* ocular lesions from false negative substances in the BCOP test method using the EPA classification system. 2010. Last Updated. http://ntp.niehs.nih.gov/iccvam/docs/ocutox_docs/invitro-2010/body.pdf Date Accessed 12 7, 2016.
46. Perkin Elmer Informatics. *Calculation of magnesium carbonate formula weight*. ChemBioDraw. Version. 2016;11.
47. National Library of Medicine (NLM). *Toxnet Databases. Hazardous Substances Data Bank. File on Magnesium Carbonate*;2016. Last Updated https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm Date Accessed 3 11, 2016.