Safety Assessment of Beta-Alanine Diacetic Acid and Tetrasodium Glutamate Diacetate as Used in Cosmetics

Status:	Scientific Literature Review for Public Comment
Release Date:	July 19, 2019
Panel Meeting Date:	December 9-10, 2019

All interested persons are provided 60 days from the above release date (September 17, 2019) to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.

The 2019 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer.

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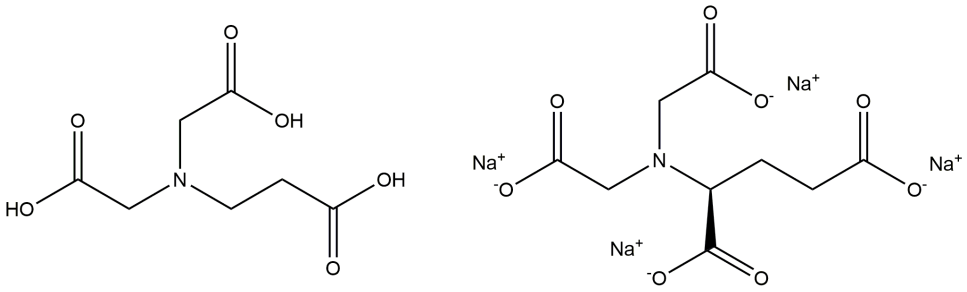
INTRODUCTION

Beta-Alanine Diacetic Acid and Tetrasodium Glutamate Diacetate are reported to function in cosmetics as chelating agents, according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*; see Table 1).[1] These ingredients are both *N,N*-diacetate-substituted amino acids. This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that CIR typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

CHEMISTRY

Definition

The definitions of the ingredients included in this review are provided in Table 1.[1] Beta-Alanine Diacetic Acid and Tetrasodium Glutamate Diacetate both function as chelating agents in cosmetic formulations. The structures of these *N,N*-diacetate-substituted amino acids are depicted in Figure 1.



Beta-Alanine Diacetic Acid **Tetrasodium Glutamate Diacetate**

Figure 1. Amino Acid Diacetates

Physical and Chemical Properties

Available physical and chemical properties of Beta-Alanine Diacetic Acid and Tetrasodium Glutamate Diacetate are provided in Table 2.[2-4] Tetrasodium Glutamate Diacetate is an odorless white to off-white powder that is very soluble in water (650 g/l).[2]

Method of Manufacture

No methods of manufacture were found in the public literature, and unpublished data were not provided.

Composition/Impurities

No composition or impurities data were found in the public literature, and unpublished data were not provided.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (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 the FDA Voluntary Cosmetic Registration Program (VCRP) database. Data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2019 VCRP survey data, Tetrasodium Glutamate Diacetate is used in a total of 794 formulations; the majority of the uses are in bath soaps and detergents (Table 3).[5] Beta-Alanine Diacetic Acid is reported to be used in only 2 leave-on formulations: a moisturizing skin care product and "other" hair preparations. The results of the concentration of use survey conducted by the Council in 2018 indicate that Tetrasodium Glutamate Diacetate is used at up to 1%; this concentration is reported for deodorants (non-spray).[6] No concentrations of use were reported for Beta-Alanine Diacetic Acid.

Tetrasodium Glutamate Diacetate may be used in products that can come into contact with the eyes or mucous membranes; for example, it is reported to be used in eyeliner at up to 0.057% and in bath soaps and detergents at up to 0.28%.[6] Additionally, Tetrasodium Glutamate Diacetate is used in cosmetic sprays and could possibly be inhaled; for example, it is

reported to be used at up to 0.029% in hair spray.[6] 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 < 10 µm compared with pump sprays.[7,8] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[9,10]

Beta-Alanine Diacetic Acid and Tetrasodium Glutamate Diacetate are not restricted from use in any way under the rules governing cosmetic products in the European Union.[11]

TOXICOKINETIC STUDIES

Absorption, Distribution, Metabolism, and Elimination (ADME)

Oral

Tetrasodium Glutamate Diacetate

In a single dose elimination study, Wistar rats (4 per sex per group) received Tetrasodium Glutamate Diacetate (87.3% pure) in water via gavage at 100, 300, or 1000 mg/kg.[2] Most of the test material was found in the feces, with an overall recovery ranging from 95.8% – 103.0%. No further details were provided

In a 90-day elimination study, groups of 10 male and 10 female Wistar rats received 0, 100, 300, or 1000 mg/kg bw Tetrasodium Glutamate in water via gavage daily.[2] Concentrations of the test material in the urine were below the detection limit (< 50 mg/kg urine) in the control, low-, and mid-dose groups at the end of treatment. The researchers determined that absorption from the gastrointestinal tract was low. No further details were provided.

Intraperitoneal

Tetrasodium Glutamate Diacetate

In a single dose elimination study, Wistar rats (4 per sex per group) received Tetrasodium Glutamate Diacetate (87.3% pure) in water via intraperitoneal administration at 5, 15, or 50 mg/kg.[2] The test material was mainly detected in urine (83% of the animals), with an overall recovery ranging from 74.6% – 103.3%. The results indicated that Tetrasodium Glutamate Diacetate is excreted unmetabolized. No further details were provided.

TOXICOLOGICAL STUDIES

Acute Toxicity

Acute toxicity data is summarized in Table 4.[2] The acute dermal and oral LD_{50}s for Tetrasodium Glutamate Diacetate in rats were greater than 2000 mg/kg bw. The LC_{50} for an inhalation study of Tetrasodium Glutamate Diacetate was greater than 4.2 mg/l in rats.

Repeated Dose Toxicity

The potential adverse effects of 95% Tetrasodium Glutamate Diacetate was investigated in a 90-day oral toxicity study in specific pathogen-free (SPF) Wistar rats.[2] The study was performed in accordance with Organization for Economic Co-operation and Development (OECD) test guideline (TG) 408. Groups of 10 male and 10 female rats received 0, 100, 300, or 1000 mg/kg /day of the test material via gavage. An extra 10 animals per sex were used for the control and high dose groups, to assess recovery for 14 days. No treatment-related changes were observed in clinical appearance, functional observations, body weight gains, and feed consumption at up to 1000 mg/kg/day. At 1000 mg/kg/day, an increased red blood cell count was observed in males, a reduced mean corpuscular volume and hemoglobin were observed in both males and females, and increased red blood cell distribution width and increased platelet count were observed in females. A reduced mean corpuscular hemoglobin was also observed in males and females of the 300 mg/kg/day group. Changes in clinical biochemistry parameters at 1000 mg/kg/day at the end of treatment included increased albumin and cholesterol levels (males and females, respectively), reduced creatinine levels (both males and females), and reduced inorganic phosphate and chloride levels (males and females, respectively). Changes in blood chemistry were within, or just outside, the range considered normal for rats of this age and strain, and had resolved by the end of the recovery period.

Urinalysis reported an increased sodium concentration/excretion in males and females at 300 and 1000 mg/kg/day, reduced urinary volume and clarity in females, and increased specific gravity, protein level, and potassium concentration in females. These changes were absent at the end of the recovery period, indicating that these were reversible in nature. Slightly increased kidney weights and kidney-to-body weight ratios were observed in males at 1000 mg/kg/day at the end of the treatment phase. At the end of the recovery phase, kidney weights were similar to control values. In females of the 1000 mg/kg/day dose group, kidney weights and kidney-to-body weight ratios were not affected at the end of the treatment period, but were increased at the end of the recovery period, indicating that the test material had an effect on kidney function. No other toxicologically significant changes were noted during macroscopic and microscopic examination. The no-observed-adverse-effect-level (NOAEL) for Tetrasodium Glutamate Diacetate in this study was 300 mg/kg/day.[2]

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Oral

Tetrasodium Glutamate Diacetate

The effects of Tetrasodium Glutamate Diacetate on reproduction was assessed in a two-generation study using groups of 24 male and 24 female Wistar Han rats.[2] Based on the results of the dose-range-finding study, dose levels for the main study were 0, 1500, 5000, and 15,000 ppm of Tetrasodium Glutamate Diacetate in feed. A second high-dose level group received the test material in feed that was supplemented with 1000 ppm zinc carbonate to compensate for potential effects from the chelating properties of the test material. The F_0 males and females were exposed to the test material from 10 weeks prior to mating, and exposure was continued until euthanasia (males) or one day before euthanasia (females). F_0 females were allowed to produce and rear a litter until Day 21 of lactation. On Day 4 of lactation, litters were reduced in size to 8 pups (4 per sex) by random culling of F_1 pups. After weaning, one F_1 male and one F_1 female of each litter of each dose group (except the high dose zinc supplemented group) were selected for mating with a pup of another litter of the same dose group to produce an F_2 generation.

The F_1 adults were dosed in the same manner as the F_0 adults, except there was no zinc supplement group. After weaning, animals were treated for a minimum of 70 days prior to mating and continuing until euthanasia (males) or one day before euthanasia (females). F_1 females were allowed to produce and rear a litter until Day 21 of lactation. On Day 4 of lactation, litters were reduced in size to 8 pups by random culling of F_2 pups. During the study, the rats were evaluated for mortality, clinical signs of toxicity, body weights, feed consumption, clinical laboratory investigations (including collection of blood samples for possible future zinc analysis; females only), reproduction processes, observations on offspring, gross lesions, skeletal examination of offspring, organ weights, and histopathology.

No significant adverse effects were observed in parental animals or with reproduction or development in the 1500 ppm or 5000 ppm dose groups. At 15,000 ppm, with and without zinc, an increase in mean kidney weight was observed in F_0 and F_1 adults, and slight histopathological renal changes were observed in F_1 adults. The renal changes were minor and consisted of an increase in cortical tubular dilation in females and an increase of corticomedullary tubular basophilia in males. No significant adverse effects were observed with reproduction or development in the 15,000 ppm dose group. Based on these findings, the parental NOAEL was determined to be 5000 ppm, and the reproductive and developmental NOAELs were determined to be 15,000 ppm.[2]

In an oral developmental toxicity study of Tetrasodium Glutamate Diacetate, groups of 22 female Wistar Han rats received the test material in water via gavage at doses of 0 or 1000 mg/kg bw/day on Day 6 through Day 20 of gestation.[2] The animals were checked daily for clinical signs of toxicity. Body weights and water and feed consumption were determined at periodic intervals. All animals surviving to Day 20 of gestation were necropsied, and external, thoracic, and abdominal macroscopic findings were recorded. The uteri and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Uterine weights were recorded. Viable fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations. No adverse effects considered to be treatment-related were observed in either the dams or the fetuses. The maternal and developmental NOAEL was considered to be 1000 mg/kg bw/day Tetrasodium Glutamate Diacetate.

In an oral developmental toxicity study, Tetrasodium Glutamate Diacetate (87.3%) in water was given to groups of 22 female New Zealand White rabbits.[2] The rabbits received the test material at 0, 20, 75, or 300 mg/kg via gavage daily from Day 7 to Day 28 of gestation. The animals were checked daily for clinical signs of toxicity. Body weights and water and feed consumption were determined at periodic intervals. All animals surviving to Day 29 of gestation were necropsied and macroscopic findings were recorded. A laparohysterectomy was performed on each surviving female of the groups. The uteri, placenta, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and corrected body weights were calculated. The fetuses were weighed, sexed, and examined for malformations and developmental variations. All live fetuses were killed and examined for visceral anomalies.

One dam of the 20 mg/kg dose group died on Day 22 of gestation due to gavage error. No maternal toxicity was observed in the 20 mg/kg dose group. In animals treated with 75 mg/kg bw, dark feces, diarrhea, reduced feces production, and slightly reduced feed and water intake were also observed; however, these changes were very limited and in view of the absence of more severe effects, such as changes in body weight gains, these effects were not considered to be toxicologically relevant. In dams at the 300 mg/kg dose level, clinical signs of toxicity consisted of increased incidences of dark feces, diarrhea, and reduced feces production. Feed and water consumption were reduced. Body weight gains were decreased, with several animals showing a transient body weight loss. No developmental toxicity was observed in the 20, 75, and 300 mg/kg/day groups. Based on the results of this study, the maternal no-observed-effect-level (NOEL) for Tetrasodium Glutamate Diacetate was determined to be 20 mg/kg body weight/day; the maternal NOAEL was determined to be 75 mg/kg body weight/day. The developmental NOAEL was at least 300 mg/kg body weight/day.[2]

In a similar developmental study in inseminated female New Zealand White rabbits, groups of 24 animals received 0, 30, 100, or 300 mg/kg Tetrasodium Glutamate Diacetate in water once daily by gavage from Days 7 to 28 of gestation.[2] A

second high-dose level group received the test material in feed that was supplemented with 1024 ppm zinc carbonate to compensate for potential effects from the chelating properties of the test material. Dose-dependent, treatment-related clinical signs that consisted of an increased incidence of dark feces and reduced feces production were observed in the 100, 300, and 300 + zinc dose groups. Body weights and/or body weight gain were reduced at 300 mg/kg (with and without zinc) throughout most of the treatment period. Feed consumption was decreased at 100 mg/kg, 300 mg/kg, and 300 mg/kg + zinc in a dose-dependent manner for the first one or two weeks of treatment. No effect on water consumption was noted. No treatment-related effects were seen in hematology parameters up to 300 mg/kg without added zinc. No treatment- related effects on clinical biochemistry and urinalysis parameters were noted. There were no treatment-related macroscopic findings. No effects were noted on the number of corpora lutea, implantation sites, viable or dead fetuses, early or late resorptions, pre- and post-implantation loss, litter size, and sex ratio. There were no significant differences in fetal body weight following treatment up to 300 mg/kg without added zinc. The addition of dietary zinc to animals treated with 300 mg/kg bw/day resulted in additional maternal toxicity (hematological changes) and in fetal toxicity (reduced fetal body weights). The maternal NOAEL for Tetrasodium Glutamate Diacetate was determined to be 30 mg/kg bw/day; the developmental NOAEL was determined to be at least 300 mg/kg bw/day.[2]

GENOTOXICITY

Genotoxicity studies are summarized in Table 5. Tetrasodium Glutamate Diacetate (70.7%) was neither genotoxic with or without metabolic activation in an Ames test at up to 5000 µg/plate nor in a Chinese hamster ovary gene mutation assay at up to 3650 µg/ml; however, it was weakly clastogenic in a Chinese hamster lung cell chromosome aberration test at 1825 and 3650 µg/ml with or without metabolic activation.[2] No genotoxicity was observed to Tetrasodium Glutamate Diacetate (70.7%) in an in vivo mammalian erythrocyte micronucleus test in mice at up to 400 mg/kg bw.

CARCINOGENICITY

No carcinogenicity studies were found in the published literature, and unpublished data were not submitted.

IRRITATION AND SENSITIZATION

Irritation

Animal

Tetrasodium Glutamate Diacetate

The dermal irritation potential of Tetrasodium Glutamate Diacetate (purity, 70.7%) in water was assessed using three New Zealand White rabbits in accordance with OECD TG 404.[2] Application of a single 4-h, semi-occluded patch (2.5 cm^2) containing 0.5 ml test material on intact skin produced very slight erythema in all rabbits. All treated skin sites appeared normal at the 24-h observation. The test material produced a primary irritation index of 0.0 and was classified as non-irritating. No corrosive effects were observed.

Sensitization

Animal

Tetrasodium Glutamate Diacetate

In a guinea pig maximization study of a test material containing 74.33% Tetrasodium Glutamate Diacetate, 20 female Dunkin-Hartley guinea pigs received the test material in distilled water at 1% w/v during the intradermal induction, 50% w/w during the topical induction, and 50% and 25% w/w during the topical challenge.[2] Positive and negative control groups consisted of 10 animals each. No adverse skin effects were observed in the animals that received the test material. The controls yielded expected results. The test material containing 74.33% Tetrasodium Glutamate Diacetate was determined to be non-sensitizing in this study.

OCULAR IRRITATION STUDIES

In Vivo

Animal

Tetrasodium Glutamate Diacetate

The ocular irritation potential of Tetrasodium Glutamate Diacetate (purity, 70.7%) in water was assessed using three New Zealand White rabbits in accordance with OECD TG 405.[2] A single instillation of the test material (0.1 ml) to unrinsed eyes produced minimal conjunctival irritation. All treated eyes appeared normal 48 h after treatment. Tetrasodium Glutamate Diacetate was considered to be non-irritating.

SUMMARY

Beta-Alanine Diacetic Acid and Tetrasodium Glutamate Diacetate both function as chelating agents in cosmetic formulations. According to the 2019 VCRP survey data, Tetrasodium Glutamate Diacetate is used in a total of 794 formulations; the majority of the uses are in bath soaps and detergents. Beta-Alanine Diacetic Acid is reported to be used in only 2 leave-on formulations, a moisturizing skin care product and "other" hair preparations. The results of the concentration of use survey conducted by the Council in 2018 indicate that Tetrasodium Glutamate Diacetate is used at up to 1%: this concentration is reported in deodorants (non-spray). No concentrations of use were reported for Beta-Alanine Diacetic Acid.

In oral and intraperitoneal elimination studies in rats, Tetrasodium Glutamate Diacetate (at up to 1000 mg/kg bw) was mostly recovered unmetabolized in the urine and feces.

The acute dermal and oral LD_{50}s for Tetrasodium Glutamate Diacetate in rats were greater than 2000 mg/kg bw. The LC_{50} for an inhalation study of Tetrasodium Glutamate Diacetate was greater than 4.2 mg/L in rats.

The NOAEL for a 90-day oral toxicity study of 95% Tetrasodium Glutamate Diacetate was 300 mg/kg/day in rats. The rats received 0, 100, 300, or 1000 mg/kg/day daily via gavage. Slightly increased kidney weights and kidney-to-body weight ratios were observed in males with 1000 mg/kg/day at the end of the treatment phase. At the end of the recovery phase, kidney weights were similar to control levels. In females at 1000 mg/kg/day, kidney weights and kidney-to-body weight ratios were increased at the end of the recovery period, but not at the end of the treatment period.

A dietary two-generation study in rats reported no skeletal malformations at up to and including the maximum dose of 15,000 ppm. Therein, the parental NOAEL was determined to be 5000 ppm and the reproductive and developmental NOAEL was 15,000 ppm. The NOAEL for developmental and maternal toxicity in rats in an oral gavage study was 1000 mg/kg bw/day (maximum dose tested). The NOAEL for developmental toxicity in rabbits in another oral gavage study was 300 mg/kg bw/day (maximum dose tested), and the maternal NOAEL in rabbits was 75 mg/kg bw/day.

Tetrasodium Glutamate Diacetate (70.7%) was neither genotoxic with or without metabolic activation in an Ames test at up to 5000 µg/plate nor in a Chinese hamster ovary gene mutation assay at up to 3650 µg/ml; however, it was weakly clastogenic in a Chinese hamster lung cell chromosome aberration test at 1825 and 3650 µg/ml with or without metabolic activation.[2] No genotoxicity was observed to Tetrasodium Glutamate Diacetate (70.7%) in an in vivo mammalian erythrocyte micronucleus test at up to 400 mg/kg bw.

In dermal studies, Tetrasodium Glutamate Diacetate was non-irritating in rabbits and non-sensitizing (at up to 50%) in guinea pigs. Tetrasodium Glutamate was non-irritating in an ocular irritation study in rabbits.

No toxicological data were available for Beta-Alanine Diacetic Acid. No methods of manufacture, composition or impurities data, or carcinogenicity data were found in the published literature; and unpublished data were not submitted for either Beta-Alanine Diacetic Acid or Tetrasodium Glutamate Diacetate.

INFORMATION SOUGHT

The CIR is seeking information regarding physical properties, concentration of use, and toxicological data for Beta-Alanine Diacetic Acid; methods of manufacturing and composition and impurities data are needed on both Beta-Alanine Diacetic Acid and Tetrasodium Glutamate Diacetate. Additional toxicological data for Tetrasodium Glutamate Diacetate, specifically dermal irritation and sensitization data at maximum use concentrations, are especially being sought in order to help the CIR Expert Panel assess the safety of the use of these ingredients in cosmetics.

TABLES

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

Ingredient/CAS No.	Definition	Function
Beta-Alanine Diacetic Acid 6245-75-6	Beta-Alanine Diacetic Acid is the substituted amino acid that conforms to the formula in Figure 1.	Chelating Agents
Tetrasodium Glutamate Diacetate 51981-21-6	Tetrasodium Glutamate Diacetate is the organic compound that conforms to the formula in Figure 1.	Chelating Agents

Table 2. Chemical and physical properties

Property	Value	Reference
Beta-Alanine Diacetic Acid		
Molecular Weight (g/mol)	205.166	[3]
Tetrasodium Glutamate Diacetate		
Physical Form	odorless white to off-white powder	[2]
Molecular Weight (g/mol)	351.1291	[4]
Density at 20 °C	1.466	[2]
Vapor Pressure (mmHg) at 20 °C	0.600	[2]
Melting Point (°C)	280 (decomposition)	[2]
Water Solubility (g/l) at 21 °C and pH 7	650	[2]
log $P_{o/w}$ at 27 °C and pH 7	< 0	[2]

Table 3. Frequency (2019) and concentration of use (2018) according to duration and type of exposure for amino acid diacetates.[5,6]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Beta-Alanine Diacetic Acid		**Tetrasodium Glutamate Diacetate**	
Totals†	**2**	**NR**	**794**	**0.0013-1**
Duration of Use				
Leave-On	2	NR	110	0.0013-1
Rinse Off	NR	NR	684	0.037-0.31
Diluted for (Bath) Use	NR	NR	NR	NR
Exposure Type				
Eye Area	NR	NR	16	0.048-0.057
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	1[a]	NR	1; 43[a]; 35[b]	0.029; 0.033-0.094[a]
Incidental Inhalation-Powder	NR	NR	35[b]; 1[c]	0.057[c]
Dermal Contact	1	NR	759	0.0013-1
Deodorant (underarm)	NR	NR	NR	1
Hair - Non-Coloring	1	NR	19	0.029-0.097
Hair-Coloring	NR	NR	15	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	631	0.037-0.28
Baby Products	NR	NR	4	NR

NR = Not reported

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

[a.] It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.

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

[c.] It is possible these products may be powders, but it is not specified whether the reported uses are powders.

Table 4. Acute toxicity

Ingredient/Concentration/Vehicle	Dose	Species	Study Protocol	Results	Reference
		Dermal			
Tetrasodium Glutamate Diacetate (91% pure); 200 mg/ml (concentration of solution); water	2000 mg/kg bw	5 male and 5 female Wistar rats	Occlusive on back; test area 25 cm^2 for males and 18 cm^2 for females; test site washed with tap water after 24 h; in accordance with OECD TG 402; observed for 14 days	LD_{50} > 2000 mg/kg bw; no mortalities; flat and/or hunched posture, piloerection, and/or slight chromodacryorrhea noted in all animals from day 1 through day 4; slight scales and/or scabs observed in treated skin of 4 females from day 3 through day 9	[2]
		Oral			
Tetrasodium Glutamate Diacetate (70.7% pure); 200 mg/ml; in water	2000 mg/kg	5 male and 5 female Sprague-Dawley rats	Gavage; observed for 14 days	LD_{50} > 2000 mg/kg bw; no mortalities; no clinical signs of toxicity; no other abnormalities	[2]
Tetrasodium Glutamate Diacetate (tradename mixture was ~78% tetra- and trisodium salt); ~35% solution in water	560 mg/kg as tradename mixture	5 male and 5 female rats; species not described	Gavage in accordance with OECD TG 401; observed for 14 days	LD_{50} > 560 mg/kg bw; no mortalities; no clinical signs of toxicity	[2]
		Inhalation			
Tetrasodium Glutamate Diacetate (90% pure); 50% (concentration in vehicle); water; particle size range 1 - 4 µm	4.2 mg/l (4.3 mg/l was technically the highest attainable concentration); mass median aerodynamic diameter) /geometric standard deviation were 2.8/2.7 µm	5 male and 5 female Wistar rats	Nose-only inhalation for 4 h in accordance with OECD TG 403; observed for 14 days	LC_{50} > 4.2 mg/l; no mortalities; slightly decreased breathing rate observed during exposure; soiled fur observed after exposure until day 2; sniffing noted in 4 animals shortly after exposure, in 7 animals on day 1, and in 1 animal on day 2; eye discharge noted in 1 animal on day 1	[2]

Table 5. Genotoxicity studies

Ingredient/Concentration	Dose	Species/Strain/Cell	Method	Results	Reference
		In Vitro			
Tetrasodium Glutamate Diacetate (70.7%) in distilled water	Up to 5000 µg/plate, with or without metabolic activation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538	Ames test	Not genotoxic	[2]
Tetrasodium Glutamate Diacetate (70.7%) in distilled water	228 - 3650 µg/ml, with or without metabolic activation	Chinese hamster ovary	HGPRT locus on X-chromosome gene mutation assay	Not genotoxic	[2]
Tetrasodium Glutamate Diacetate (70.7%) in minimal essential media (MEM)	228 - 3650 µg/ml, with or without metabolic activation	Chinese hamster lung cell line	Chromosome aberration test	Weakly clastogenic; small but statistically significant increases in the frequency of cells with aberrations were observed in cells exposed for 6-h with and without metabolic activation and in the 48-h (without metabolic activation) continuous exposure groups; test material was shown to be toxic	[2]
		In Vivo			
Tetrasodium Glutamate Diacetate (70.7%) in distilled water	0, 100, 200, or 400 mg/kg bw	Groups of 5 male and 5 female CD-1 mice	Mammalian erythrocyte micronucleus test	Not genotoxic	[2]

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