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

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All interested persons are provided 60 days from the above release date (i.e., February 12, 2021) to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to the Cosmetic Ingredient Review (CIR) will be discussed in open meetings, will be available at the CIR office for review by any interested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 Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. Previous Panel member involved in this assessment: James G. Marks, Jr., M.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer, CIR.

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ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Beta-Alanine Diacetic Acid and Tetrasodium Glutamate Diacetate, which are reported to function as chelating agents in cosmetic products. The Panel reviewed the available data to determine the safety of these ingredients. The Panel concluded that Tetrasodium Glutamate Diacetate is safe in cosmetics in the practices of use and concentration described in this safety assessment. However, the Panel also concluded that the available data are insufficient to make a determination that Beta-Alanine Diacetic Acid is safe under the intended conditions of use in cosmetic formulations.

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*).[1] These ingredients are both *N,N*-diacetate-substituted amino acids. The Expert Panel for Cosmetic Ingredient Safety (Panel) previously reviewed the safety of the α-amino acids, including alanine, glutamic acid, and sodium glutamate.[2] The Panel concluded that these ingredients are safe in the present practices of use and concentration in cosmetics. The two ingredients under review herein, are both amino acids di-substituted at the amine functional group with acetate.

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 the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (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.

Much of the data included in this safety assessment was found on the European Chemicals Agency (ECHA) website on the L-isomer of Tetrasodium Glutamate Diacetate.[3] Please note that the EHCA website provides summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when ECHA is cited.

CHEMISTRY

Definitions and Structures

Beta-Alanine Diacetic Acid (CAS No. 6245-75-6) and Tetrasodium Glutamate Diacetate (CAS No. 51981-21-6; L-isomer) both function as chelating agents in cosmetic formulations.[1] The structures of these *N,N*-diacetate-substituted amino acids are depicted in Figure 1.

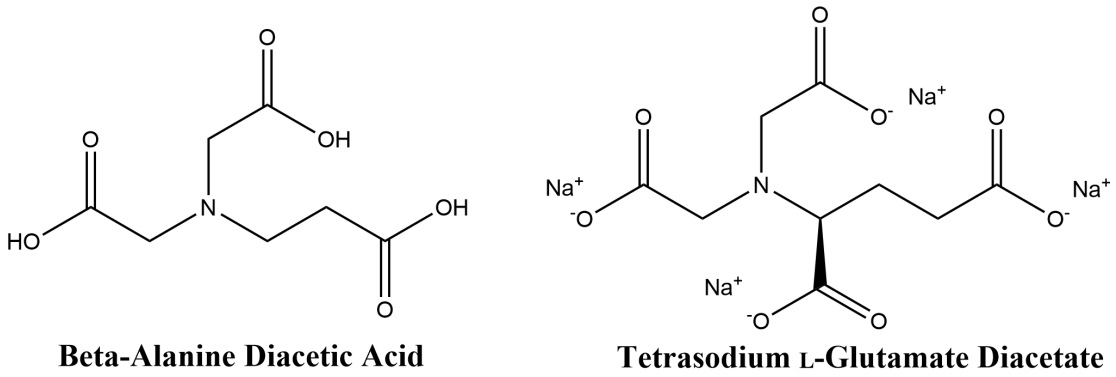

Figure 1. Amino Acid Diacetates

Chemical Properties

Available chemical properties of Beta-Alanine Diacetic Acid and Tetrasodium Glutamate Diacetate are provided in Table 1.[3-5] Tetrasodium Glutamate Diacetate is an odorless white to off-white powder that is very soluble in water (650 g/l) and has a log $P_{o/w}$ of < 0.[3] β-Alanine has no stereocenter; however, glutamic acid does and the naturally occurring form is the L-isomer. The formula weight for Tetrasodium Glutamate Diacetate is 351.13 g/mol.[5] Beta-Alanine Diacetic Acid has a molecular weight of 205.17 g/mol and an estimated log $P_{o/w}$ of -3.32.[4,6]

A supplier reports that racemization of L-Tetrasodium Glutamate Diacetate is facilitated by low pH.[7,8] At ambient temperatures, racemization takes "a very, very long time (many months)" to occur; while at 9000.74 mm Hg and 95 - 100 ºC, it takes ≥ 70 h to get full racemization. Very high temperatures using an autoclave are needed to racemize in a few hours.

Method of Manufacture

Tetrasodium Glutamate Diacetate

Figure 2 and Figure 3 describe the manufacturing processes of Tetrasodium Glutamate Diacetate by two different suppliers. In one process, neutralized monosodium glutamate and neutralized monochloroacetic acid are reacted together to produce the ingredient, while in the second process, monosodium glutamate, hydrogen cyanide, and formaldehyde are reacted together and then saponified with sodium hydroxide to produce the ingredient.

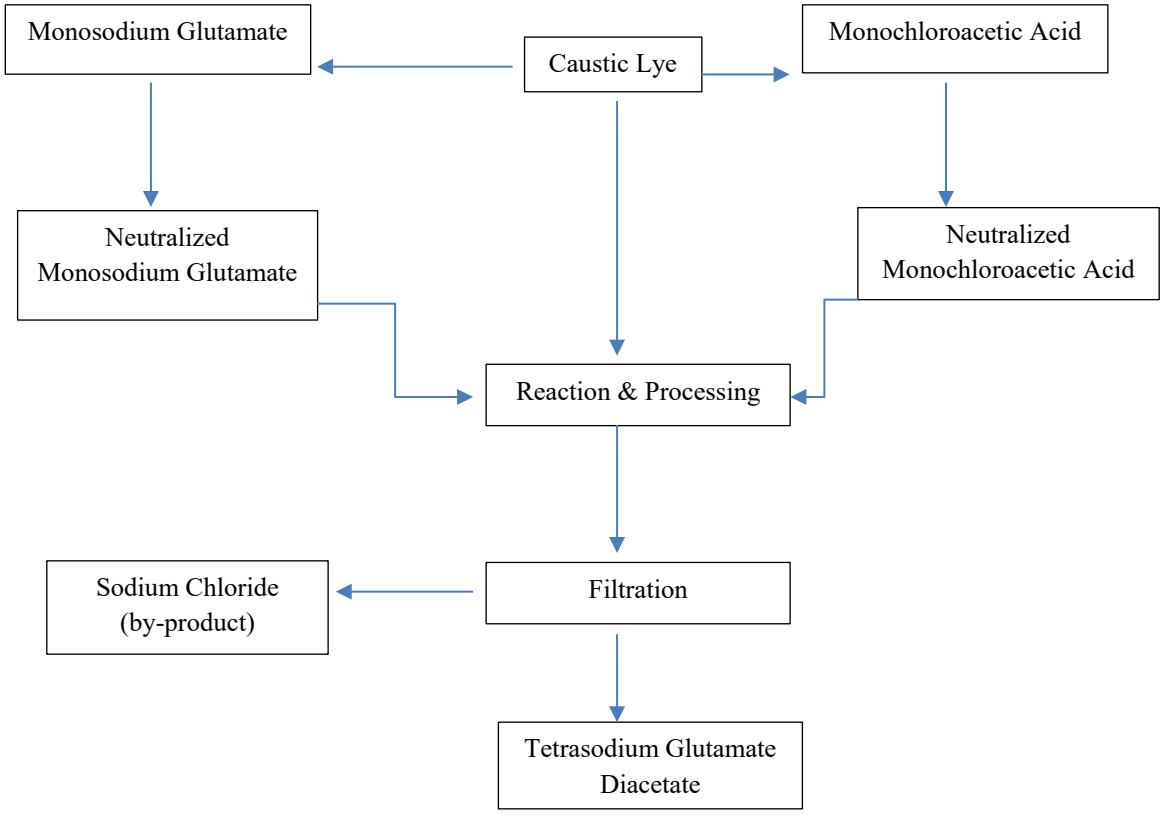

Figure 2. Manufacturing flow chart of a Tetrasodium Glutamate Diacetate tradename product.[9]

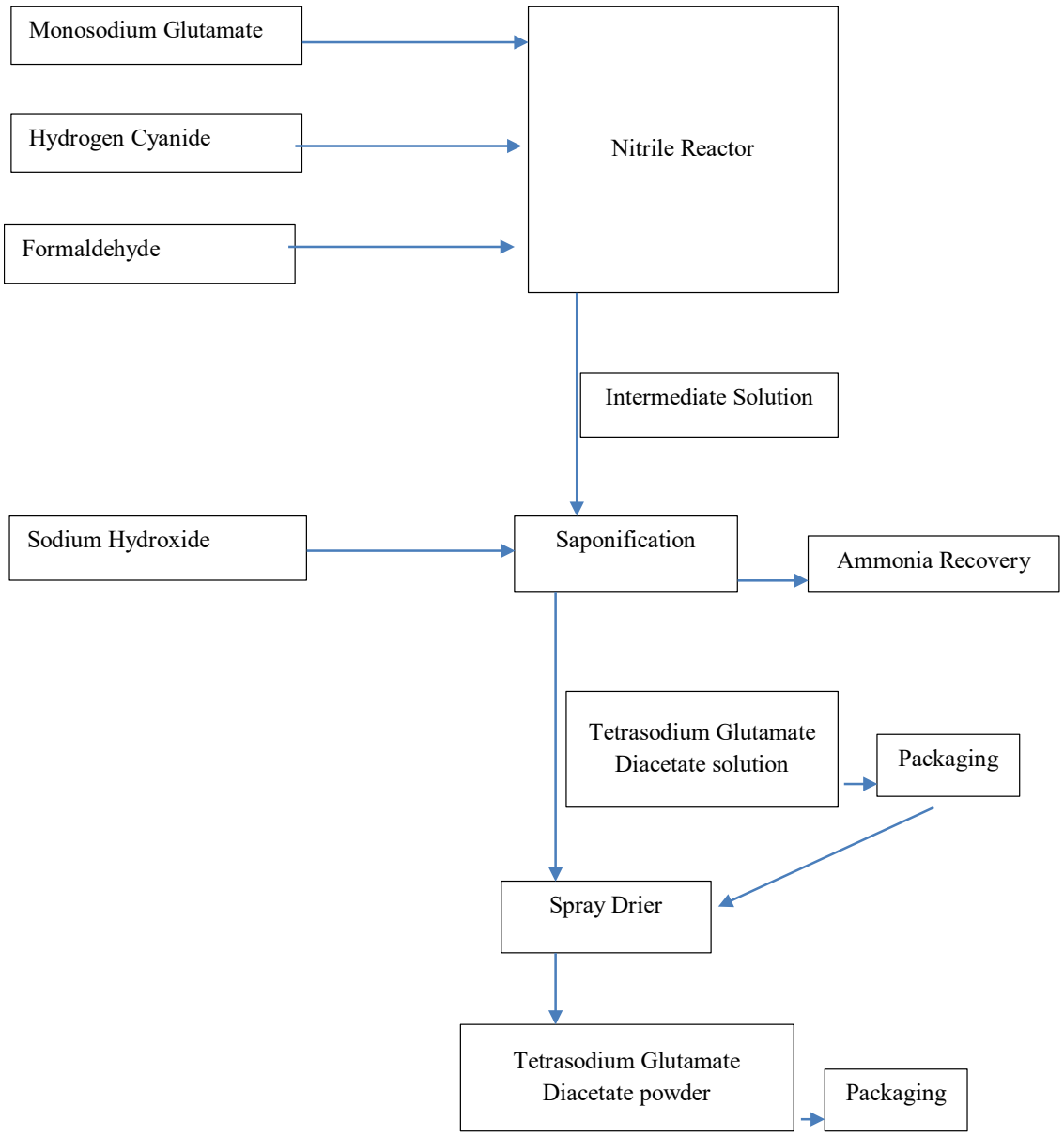

Figure 3. Manufacturing flow diagram of a Tetrasodium Glutamate Diacetate tradename solution and powder.[10]

Beta-Alanine Diacetic Acid

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

Composition/Impurities

Tetrasodium Glutamate Diacetate

A supplier of Tetrasodium Glutamate Diacetate reports that a tradename product contains 46.5% - 47.5% Tetrasodium Glutamate Diacetate, 0.00% - 0.40% sodium hydroxide, and 52.0% - 54.0% water.[11] Another supplier reports that a tradename product contains approximately 81.0% Tetrasodium Glutamate Diacetate, 1.1% sodium hydroxide (a raw material), 15.9% water, 1.7% sodium glycolate (an impurity), 0.15% sodium formate, and 0.15% nitrilotriacetic acid, trisodium salt[12] (an impurity that is a 2B carcinogen, according to the International Agency for Research on Cancer (IARC)[13]). A trace metals report from this same supplier details the following heavy metals profile: arsenic < 5 mg/kg, cadmium < 0.5 mg/kg, chromium < 1 mg/kg, lead < 5 mg/kg, mercury < 0.05 mg/kg, and nickel < 5 mg/kg.[14]

Beta-Alanine Diacetic Acid

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 2020 VCRP survey data, Tetrasodium Glutamate Diacetate is used in a total of 977 formulations; the majority of the uses are in bath soaps and detergents (Table 2).[15] 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).[16] 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%.[16] 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.[16] 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.[17,18] 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.[19,20]

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.[21]

TOXICOKINETIC STUDIES

Dermal Penetration

No dermal penetration data were found in the public literature, and unpublished data were not provided.

Absorption, Distribution, Metabolism, and Excretion (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.[3] Most of the test material was found in the feces, with an overall recovery ranging from 95.8% – 103.0%. The duration of follow-up was 72 h. More than 85% of the dose was excreted in the first 24 h, unmetabolized.

In a 90-d 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.[3] 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 (i.p.) administration at 5, 15, or 50 mg/kg.[3] In 83% of the animals, the test material was mainly detected in urine, with an overall recovery ranging from 74.6% – 103.3%. (Details regarding excretion by the remaining animals were not provided.) More than 85% of the dose was excreted in the first 24 h. The results indicated that Tetrasodium Glutamate Diacetate is excreted unmetabolized.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Acute toxicity studies summarized here are described in Table 3.[3] The acute dermal and oral LD_{50}s for Tetrasodium Glutamate Diacetate (purity ranging from 70.7% to 91%) 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.

Subchronic Toxicity Studies

Oral

Tetrasodium Glutamate Diacetate

The potential adverse effects of 95% Tetrasodium Glutamate Diacetate was investigated in a 90-d oral toxicity study in specific pathogen-free (SPF) Wistar rats.[3] 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/d 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 d. No treatment-related changes were observed in clinical appearance, functional observations, body weight gains, and feed consumption at up to 1000 mg/kg/d. At 1000 mg/kg/d, 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/d group. Changes in clinical biochemistry parameters at 1000 mg/kg/d 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/d. At 1000 mg/kg/d in females, reduced urinary volume and clarity, and increased specific gravity, protein level, and potassium concentration was observed. 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/d 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/d 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/d.[3]

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

Tetrasodium Glutamate Diacetate

The effects of Tetrasodium Glutamate Diacetate on reproduction were assessed in a two-generation study using groups of 24 male and 24 female Wistar Han rats.[3] 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 wk 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 d 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 on 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.[3]

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/d on day 6 through day 20 of gestation.[3] 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/d 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.[3] 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/d 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/d; the maternal NOAEL was determined to be 75 mg/kg body weight/d. The developmental NOAEL was at least 300 mg/kg body weight/d.[3]

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.[3] 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/d 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/d; the developmental NOAEL was determined to be at least 300 mg/kg bw/d.[3]

GENOTOXICITY STUDIES

Genotoxicity studies summarized here are described in Table 4. Tetrasodium Glutamate Diacetate (70.7%) was not genotoxic, with or without metabolic activation, in an Ames test at up to 5000 µg/plate, or 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.[3] No genotoxicity was observed with Tetrasodium Glutamate Diacetate (70.7%) in an in vivo mammalian erythrocyte micronucleus test in mice at up to 400 mg/kg bw.

CARCINOGENICITY STUDIES

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

IRRITATION AND SENSITIZATION STUDIES

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.[3] 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.

Human

Tetrasodium Glutamate Diacetate

The dermal irritation potential of a liquid eyeliner containing 0.2315% Tetrasodium Glutamate Diacetate was tested using 15 subjects.[22] The subjects received an occlusive patch for 24 h with the test material at full strength. No significant differences in irritancy were observed between the test material and the reference control.

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.[3] 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.

Human

Tetrasodium Glutamate Diacetate

The dermal sensitization potential of a liquid eyeliner containing 0.2315% Tetrasodium Glutamate Diacetate was tested in a human repeat insult patch test (HRIPT) using 104 subjects. The subjects were induced with 9 occlusive patches (2 cm^2) containing 0.2 ml of the test material for 3 consecutive weeks. Following a 2-wk rest period, the subjects were challenged in previously unexposed skin for 24 h and the test sites were observed for reactions at 24 and 48 h post patch removal. No adverse effects were observed. The test material was considered non-sensitizing.[23]

OCULAR IRRITATION STUDIES

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.[3] 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 2020 VCRP survey data, Tetrasodium Glutamate Diacetate is used in a total of 977 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 an oral, single-dose elimination study with rats, Tetrasodium Glutamate Diacetate (at up to 1000 mg/kg bw) was mostly recovered unmetabolized in feces; while in an oral 90-d elimination study, concentrations of the test material in the urine were below the detection limit, and absorption from the gastrointestinal tract was low. Tetrasodium Glutamate Diacetate was mainly excreted unmetabolized in urine, in rats, in an i.p. single-dose elimination study at up to 50 mg/kg bw.

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-d oral toxicity study of 95% Tetrasodium Glutamate Diacetate was 300 mg/kg/d in rats. The rats received 0, 100, 300, or 1000 mg/kg/d daily via gavage. Slightly increased kidney weights and kidney-to-body weight ratios were observed in males with 1000 mg/kg/d 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/d, 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 a gavage study was 1000 mg/kg bw/d (only dose tested). The NOAEL for developmental toxicity in rabbits in a gavage study with Tetrasodium Glutamate Diacetate

(87.3%) in water was 300 mg/kg bw/d (maximum dose tested), and the maternal NOAEL in rabbits was 75 mg/kg bw/d. In a developmental study of inseminated female rabbits that received up to 300 mg/kg Tetrasodium Glutamate Diacetate, with and without a zinc supplement, the maternal NOAEL was 30 mg/kg bw/d and the developmental NOAEL was at least 300 mg/kg bw/d.

Tetrasodium Glutamate Diacetate (70.7%) was not genotoxic, with or without metabolic activation, in an Ames test at up to 5000 µg/plate, or 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.[3] 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 animal studies, Tetrasodium Glutamate Diacetate was non-irritating in rabbits and non-sensitizing (at up to 50%) in guinea pigs. A liquid eyeliner containing 0.2315% Tetrasodium Glutamate Diacetate was not irritating or sensitizing in human patch studies. Tetrasodium Glutamate Diacetate was non-irritating in an ocular irritation study in rabbits.

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

DISCUSSION

Beta-Alanine Diacetic Acid and Tetrasodium Glutamate Diacetate are reported to function in cosmetics as chelating agents. These ingredients are both *N,N*-diacetate-substituted amino acids.

Tetrasodium Glutamate Diacetate is reported to be used in spray and powder products that could possibly be inhaled. For example, this ingredient is used in hair spray at up to 0.029%. The Panel noted that in aerosol products, 95% – 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns (e.g., limited data available from inhalation studies, including acute exposure data, suggest little potential for respiratory effects at relevant doses). 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 https://www.cir-safety.org/cir-findings.

The Panel found that the systemic toxicity data, including developmental and reproductive toxicity studies, acute and subchronic toxicity studies, and dermal irritation and sensitization studies in this report were sufficient for assessing safety for reported cosmetic uses of Tetrasodium Glutamate Diacetate. The Panel noted that Tetrasodium Glutamate Diacetate is slowly absorbed through the gastrointestinal tract, and, dermal absorption is likely to be even slower. The Panel discussed the concern for the lack of carcinogenicity data and the fact that, according to a supplier, Tetrasodium Glutamate Diacetate may contain a salt of nitrilotriacetic acid, a 2B carcinogen according to the IARC. However, because multiple negative genotoxicity studies (in vitro and in vivo) were available, and due to the fact that concentrations of use for Tetrasodium Glutamate Diacetate in leave-on products are low, these concerns were mitigated.

The Panel noted multiple gaps in the available safety data for Beta-Alanine Diacetic Acid in this safety assessment. Because of critical structural differences, read-across from the available data on Tetrasodium Glutamate Diacetate to Beta-Alanine Diacetic Acid cannot be made. The Panel also noted that Beta-Alanine Diacetic Acid is structurally similar to nitrilotriacetic acid, which is a kidney and bladder carcinogen and a skin, eye, and respiratory tract irritant that can be absorbed orally. The Panel, however determined that read-across between these two chemicals is not appropriate due to different chelation properties. Thus, the Panel concluded that the data are insufficient to determine safety for Beta-Alanine Diacetic Acid. The additional data needed to determine safety for this cosmetic ingredient are:

- Method of manufacturing
- Composition and impurities
- Concentration of use
- Dermal irritation and sensitization data at maximum use concentration
- 28-day dermal toxicity data
    - If positive, developmental and reproductive toxicity and genotoxicity data

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Tetrasodium Glutamate Diacetate is safe in cosmetics in the present practices of use and concentration described in this safety assessment. However, the Panel also concluded that the available data are insufficient to make a determination that Beta-Alanine Diacetic Acid* is safe under the intended conditions of use in cosmetic formulations.

**There are currently no uses reported for this ingredient.*

TABLES

Table 1. Chemical properties

Property	Value	Reference
Beta-Alanine Diacetic Acid		
Molecular Weight (g/mol)	205.166	[4]
log $P_{o/w}$	-3.32 (estimated)	[6]
Tetrasodium Glutamate Diacetate		
Physical Form	odorless white to off-white powder	[3]
Formula Weight (g/mol)	351.1291	[5]
Density (at 20 °C)	1.466	[3]
Vapor Pressure (mmHg; at 20 °C)	0.600	[3]
Melting Point (°C)	280 (decomposition)	[3]
Water Solubility (g/l; at 21 °C and pH 7)	650	[3]
log $P_{o/w}$ (at 27 °C and pH 7)	< 0	[3]

Table 2. Frequency (2020) and concentration of use (2018) according to duration and type of exposure for amino acid diacetates.[15,16]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Beta-Alanine Diacetic Acid		**Tetrasodium Glutamate Diacetate**	
Totals†	**2**	**NR**	**977**	**0.0013-1**
Duration of Use				
Leave-On	2	NR	118	0.0013-1
Rinse Off	NR	NR	859	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	NR; 46[a]; 41[b]	0.029; 0.033-0.094[a]
Incidental Inhalation-Powder	NR	NR	41[b]; 1[c]	0.057[c]
Dermal Contact	1	NR	933	0.0013-1
Deodorant (underarm)	NR	NR	NR	1
Hair - Non-Coloring	1	NR	25	0.029-0.097
Hair-Coloring	NR	NR	18	NR
Nail	NR	NR	NR	NR
Mucous Membrane	NR	NR	666	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 that these products are sprays or powders, 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 3. Acute toxicity studies

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 d	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	[3]
		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 d	LD_{50} > 2000 mg/kg bw; no mortalities; no clinical signs of toxicity; no other abnormalities	[3]
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 d	LD_{50} > 560 mg/kg bw; no mortalities; no clinical signs of toxicity	[3]
		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 d	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	[3]

Table 4. 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	[3]
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	[3]
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	[3]
		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 via single intraperitoneal injection; test performed in accordance with OECD TG 474; bone marrow harvests were made at 24, 48, or 72 h post-exposure; cyclophosphamide was a positive control	Not genotoxic	[3]

REFERENCES

1. Nikitakis J, Kowcz A. Web-Based International Cosmetic Ingredient Dictionary and Handbook. http://webdictionary.personalcarecouncil.org/jsp/Home.jsp. Washington, DC: Personal Care Products Council. Last Updated: 2020. Accessed: 08/19/2020.

2. Burnett CL, Heldreth B, Bergfeld WF, et al. Safety Assessment of α-Amino Acids as Used in Cosmetics. *Int J Toxicol.* 2013;32:41S-64S.

3. European Chemicals Agency (ECHA). Tetrasodium N,N-bis(carboxylatomethyl)-L-glutamate. https://echa.europa.eu/registration-dossier/-/registered-dossier/2174/. 2019. Accessed. 07/01/2019.

4. National Center for Biotechnology Information. PubChem Database CID=535795. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/N-_2-Carboxyethyl_iminodiacetic-acid Accessed. July 12, 2019.

5. National Center for Biotechnology Information. PubChem Database CID=44630158. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/Tetrasodium-glutamate-diacetate Accessed. July 12, 2019.

6. US Environmental Protection Agency (EPA). Estimation Programs Interface Suite for Microsoft Windows, v. 4.11. Washington, DC: US EPA; 2019.

7. Nouryon. 2020. Statement regarding racemization of Tetrasodium Glutamate Diacetate.

8. Nouryon. 2020. Clarification - Tetrasodium Glutamate Diacetate.

9. Jarchem Industries I. 2019. Manufacturing flow chart Biopure™ GLDA (Tetrasodium Glutamate Diacetate).

10. Nouryon. 2019. Flow diagram/production of Dissolvine GL-47-S an GL-PD-S (Tetrasodium Glutamate Diacetate).

11. Jarchem Industries I. 2019. Composition statement Biopure™ GLDA (Tetrasodium Glutamate Diacetate).

12. Nouryon. 2020. Dissolvine GL-PD-S (Tetrasodium Glutamate Diacetate) Composition.

13. International Agency for Research on Cancer (IARC). Nitrilotriacetic Acid and its Salts. Lyon, France: International Agency for Research on Cancer;1990.

14. Nouryon. 2020. Trace metals Dissolvine GL-PD-S (Tetrasodium Glutatmate Diacetate).

15. US Food and Drug Administration (FDA) Center for Food Safety & Applied Nutrition (CFSAN). 2020. Voluntary Cosmetic Registration Program (VCRP) - Frequency of Use of Cosmetic Ingredients. College Park, MD

16. Personal Care Products Council. 2018. Concentration of Use by FDA Product Category: Tetrasodium Glutamate Diacetate and Beta-Alanine Diacetic Acid.

17. Johnsen M. The Influence of Particle Size. *Spray Technology and Marketing.* 2004;14(11):24-27.

18. Rothe H. Special Aspects of Cosmetic Spray Evaluation.

19. Bremmer H, Prud'homme de Lodder L, Engelen J. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. Bilthoven, Netherlands 2006. Netherlands National Institute for Public Health and the Environment RIVM 320104001/2006. http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Accessed 8/24/2011. Pages 1-77.

20. 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.

21. European Union. 2009. Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products

22. Anonymous. 2018. Clinical evaluation report: Human patch test (eyeliner containing 0.2315% Tetrasodium Glutamate Diacetate).

23. TKL Research. 2019. Repeated insult patch test (eyeliner containing 0.2315% Tetrasodium Glutamate Diacetate).