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Safety Assessment of Polyaminopropyl Biguanide (Polyhexamethylene Biguanide Hydrochloride) as Used in Cosmetics

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Abstract

The Expert Panel for Cosmetic Ingredient Safety (Panel) reviewed the safety of Polyaminopropyl Biguanide (polyhexamethylene biguanide hydrochloride), which functions as a preservative in cosmetic products. The Panel reviewed relevant data relating to the safety of this ingredient and concluded that Polyaminopropyl Biguanide is safe in cosmetics in the present practices of use and concentration described in the safety assessment, when formulated to be nonirritating and nonsensitizing, which may be based on a quantitative risk assessment or other accepted methodologies. The Panel also concluded that the data are insufficient to determine the safety of Polyaminopropyl Biguanide in products that may be incidentally inhaled.

Keywords

polyaminopropyl biguanide, polyhexamethylene biguanide hydrochloride, safety, cosmetics

Introduction

The safety of the cosmetic ingredient identified by the International Nomenclature of Cosmetic Ingredients (INCI) name Polyaminopropyl Biguanide is reviewed in this assessment.[1] Polyaminopropyl Biguanide is reported to be used as a preservative in cosmetics, according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*). The *chemical name* that corresponds to the cosmetic ingredient is polyhexamethylene biguanide hydrochloride (PHMB HCl), and it is the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units; it has a 6-carbon chain in each monomeric repeat unit, and is always supplied as the hydrochloride salt. International Nomenclature of Cosmetic Ingredients nomenclature often differs from standard chemical naming conventions; therefore, it should be noted that the substance identified by the *chemical name* polyaminopropyl biguanide is not a cosmetic ingredient.

In Cosmetic Ingredient Review (CIR) safety assessments, it is standard procedure to capitalize INCI names, but to use lower case for standard chemical names. Accordingly, throughout this report, when the INCI name Polyaminopropyl Biguanide is used (with appropriate capitalization), it is to be understood that it is referring to the chemical polyhexamethylene biguanide hydrochloride, and this is the ingredient with reported uses in cosmetics. Furthermore, most of the safety test data included in this report are on the chemical PHMB HCl, as indicated by the use of the INCI name. The only exception to the exclusive use of the INCI name Polyaminopropyl Biguanide in this safety assessment relates to the summary of a cytotoxicity study, in which results for PHMB and polyaminopropyl biguanide are compared.

This safety assessment includes relevant published and unpublished data for each end point that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A list of the typical search engines and websites used, sources explored, and end points that CIR evaluates, is available 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.

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Chemistry

Definition and General Characterization

Polyaminopropyl Biguanide (CAS Numbers: 32289-58-0 [PHMB HCl]; 27083-27-8 [PHMB HCl]; 28757-47-3 [PHMB]) is the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units (PHMB HCl). According to the *Dictionary*, it is the organic compound that conforms to the formula depicted in Figure 1.[1]

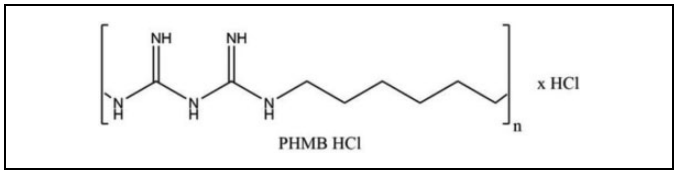

Figure 1. Polyaminopropyl biguanide (PHMB HCl).

Comments on the identity of Polyaminopropyl Biguanide were received from a chemical supplier, which stated that, effectively, all Polyaminopropyl Biguanide is PHMB HCl (ie, C6 alkyl chains linked together by biguanide groups), and no propyl biguanide groups are present.[2] The INCI name is an artifact of arbitrarily choosing the middle of the C6 alkyl chains to identify the polymer repeating units of the ingredient.

Chemical and Physical Properties

Polyaminopropyl Biguanide is a polymer that, in its neat form (as hydrochloride salt), is a solid/powder with purity >94.2%.[3] It is often marketed as an approximately 20% aqueous, preformulation solution. Chemical and physical properties are summarized in Table 1.

Method of Manufacture

One of the current methods for manufacturing Polyaminopropyl Biguanide is through the polycondensation of sodium dicyanamide and hexamethylenediamine (Figure 2).[4]

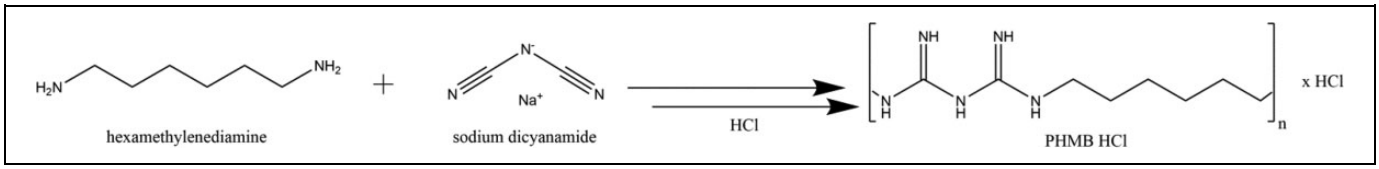

Figure 2. Synthesis of Polyaminopropyl Biguanide via the polycondensation of hexamethylenediamine and dicyanamide.

Impurities

The following chemicals have been reported as possible impurities of Polyaminopropyl Biguanide: *N*-(6-aminohexyl)-*N*′-(6-(6-guanidinohexyl)guanidine, *N*-cyano-*N*′-(6-*N*-cyanoaminohexyl)guanidine, *N*-cyano-*N*′-(6-aminohexyl)guanidine), *N*-cyano-*N*′-6-(6-guanidinohexyl)guanidine hydrochloride, and 1,6-diguanidinohexane dihydrochloride.[3]

The trace metals content (in ppm, wt/wt) of 5 different batches of technical grade Polyaminopropyl Biguanide (solid) has been reported as follows: cadmium (<0.25), chromium (<0.25-0.7), cobalt (<0.25), iron (14-40), lead (<2), zinc (370-540), arsenic (<2), and mercury (<0.2).[3]

Use

Cosmetic

The safety of Polyaminopropyl Biguanide is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database.[5] Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Council, of maximum reported use concentrations by product category.[6]

According to 2019 VCRP data, Polyaminopropyl Biguanide is being used in 147 cosmetic formulations, which are mostly leave-on products (Table 2).[5] The results of a concentration of use survey provided in 2017 indicate that Polyaminopropyl Biguanide is used at concentrations up to 0.2% in leave-on products (eye lotions), and used in baby lotions, oils, and creams (leave-on products) at concentrations up to 0.1%.[6] Polyaminopropyl Biguanide is also used at concentrations up to 0.1% in rinse-off products (ie, hair dyes and colors, and in skin cleansing products).

Cosmetic products containing Polyaminopropyl Biguanide may be applied to the skin and hair or may come in incidental contact with the eyes (at maximum use concentrations up to 0.2% in eye lotions) and mucous membranes (0.006% in "other" personal cleanliness products). Polyaminopropyl Biguanide is used in a lipstick product, the application of which may result in incidental ingestion; no concentration data were reported for this use. It is also used in baby lotions, oils, or creams at maximum use concentrations up to 0.1%. Products containing Polyaminopropyl Biguanide 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.

According to FDA VCRP data, Polyaminopropyl Biguanide is used in a fragrance preparation, which may result in incidental inhalation exposure; concentration data were not reported for this use. 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.[7-10] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) to any appreciable amount.[7,11]

Polyaminopropyl Biguanide is listed in Annex V (entry 28) of the European Commission Regulation No. 1223/2009 (Cosmetic Regulation) as a preservative to be used in all cosmetic products at a maximum concentration of 0.3%.[12] Additionally, Polyaminopropyl Biguanide is classified as CMR 2 (Carc. 2) according to the Commission Regulation (EU) No. 944/2013. CMR substances are classified as carcinogenic, mutagenic, or toxic for reproduction. A substance is placed in carcinogen Category 2 (Carc. 2, suspected human carcinogens) when the evidence obtained from human and/or animal studies is not sufficiently convincing to place the substance in Category 1A (substances known to have carcinogenic potential for humans) or Category 1B (substances presumed to have carcinogenic potential for humans). The Carc. 2 classification was effective as of January 1, 2015, and, according to Article 15 (1) of the Cosmetics Regulation, the use of Polyaminopropyl Biguanide as a cosmetic ingredient is considered to be prohibited as of this date.[3] However, Article 15 (1) of the Cosmetics Regulation also states that a substance classified in Category 2 may be used in cosmetic products if the substance has been evaluated by the Scientific Committee on Consumer Safety (SCCS) and found safe for use in cosmetic products. Accordingly, in 2017, the SCCS issued a final opinion stating that "the use of Polyaminopropyl Biguanide as a preservative in all cosmetic products at concentrations up to 0.1% is safe and that its use in sprayable formulations is not advised."

Noncosmetic

Polyaminopropyl Biguanide is reported to be the most frequently used antiseptic in traumatic and orthopedic surgery.[13] According to another source, Polyaminopropyl Biguanide has the following uses: fungicide, algicide, sanitizer in swimming pools, preservative for cut flowers, materials preservative, bacteriostat in industrial processes and water systems, and hard surface disinfectant (food and nonfood contact surfaces).[14]

Polyaminopropyl Biguanide is a broad-spectrum antimicrobial agent used in a variety of products, including contact lens cleaning solutions, skin disinfectant solutions, and wound dressings.[15] Solid wound dressings are composed of various synthetic or naturally derived materials, and typically contain added antimicrobials, such as silver, bismuth, chlorhexidine, bacitracin, or Polyaminopropyl Biguanide. Wound dressings are regulated by the US FDA as Class 1 medical devices (ie, the device is exempt from premarket notification procedures). However, this classification does not apply to wound dressings that contain added drugs, such as antimicrobial agents.[16]

Additionally, Polyaminopropyl Biguanide has been reviewed by the US Environmental Protection Agency (EPA). The EPA concluded that its use as a pesticide has very low aggregate risk of adverse health effects to the public or environment.[14]

In Australia, Polyaminopropyl Biguanide is listed in the Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons in Schedule 6.[17] Schedule 6 chemicals are described as "substances with a moderate potential for causing harm, the extent of which can be reduced through the use of distinctive packaging with strong warnings and safety directions on the label." Schedule 6 chemicals are labeled "Poison." According to this standard, Polyaminopropyl Biguanide can be used in preparations containing concentrations of 5% or less and when packed and labeled for therapeutic use.

Toxicokinetics Studies

Dermal Penetration

The dermal penetration studies summarized below are presented in Table 3.

In vitro. In one study, skin penetration experiments were performed using both rat (skin disks in solutions; 5-day equilibration phase) and human skin (receptor fluid in diffusion cell collected up to 15 days) in vitro.[18] At 0.4%, 1.4%, 5%, and 20% concentrations of Polyaminopropyl Biguanide, absorption rates through human epidermis were 8.13, 22.8, 350, and 1005 ng/cm^2/h, respectively. At 0.4%, 20% (early phase), and 20% (late phase) [^{14}C]-Polyaminopropyl Biguanide, absorption rates in rat whole skin were 131, 3695, and 11,940 ng/cm^2/h, respectively. Another study involved the application of Polyaminopropyl Biguanide (5% solution) to rat skin biopsies from newborn hairless rats and human epidermal skin in diffusion chambers. In rat skin, no absorption was detected up to day 5 of exposure. In human epidermal skin biopsies, a low rate of penetration ($\sim$ 0.09%) was noted after 24 hours. Polyaminopropyl Biguanide solutions (0.1% aqueous micellar solution, 0.1% oil-in-water emulsion, 0.3% aqueous micellar solution, and 0.3% oil-in-water emulsion) were applied to human split-thickness skin in a 2-part dermal penetration study.[3] In Part 1, penetration of the 0.1% aqueous micellar solution and 0.1% in oil-in-water emulsion was determined directly after the 24-hour exposure period. In Part 2, 24-hour exposure to the 0.3% aqueous micellar solution and to 0.3% in an oil-in-water emulsion was followed by an additional 72-hour period to determine whether the test compound that was absorbed into the skin during the previous 24-hour period would move from the skin into the receptor fluid after the washout. Skin absorption was found to be 1.56% (dermis contained 1.56% of applied dose) + 0.03% (absorbed dose = 0.03% of applied dose found in the receptor fluid). Based on SCCS Notes of Guidance, one

standard deviation (2.5%) was added to absorbed amount, yielding a calculated dermal absorption value of 4.09% (1.56% + 0.03% + 2.5% = 4.09%). After [^{14}C]-Polyaminopropyl Biguanide (tested at 0.3% wt/wt in a cosmetic formulation) was applied (occluded) to human split-thickness skin a diffusion cell, the mean absorbed dose was 0.17% at 24 hours.[18] When [^{14}C]-Polyaminopropyl Biguanide (20.2% aqueous) was applied (occluded) to human skin epidermal membranes in a diffusion cell at ~ 200 g active ingredient/L, the mean absorption percentage was 0.001% over a 24-hour period.[18] After the same test substance was warmed to 40 °C and the applied (occluded) to human skin epidermal membranes at 200 g active ingredient/L, the mean absorption percentage was 0.007% over a 24-hour period.[18]

Absorption, Distribution, Metabolism, and Excretion

The toxicokinetics studies (oral exposure) summarized below are presented in Table 4.

Animal

Oral. In rats, radiolabeled Polyaminopropyl Biguanide dosed orally was excreted principally in the feces.[3,18] In one study, rats were dosed orally with 20 mg/kg/d for 10 days and elimination after dosing was described as follows: 5.6% ± 0.35% in urine, 93.1% ± 1.58% in feces, and 0.2% exhaled.[18,19] In another animal study (male Alderley Park rats) of the distribution of radioactivity after dosing (in diet), the greatest amounts of radioactivity were detected in adipose tissue, followed by the kidneys and liver.[18,19] No radioactivity was detected in brain. Small amounts of Polyaminopropyl Biguanide oligomers with 2-cyanoguanidino end groups were found in the urine, together with trace constituents, 3,3-dicyano-1,1-hexamethylenediguanidine and a compound considered to be 1-(6-aminohexyl)-3-cyanoguanidine. Absorption was not detected in a study in which mice received a single oral dose (2 mL) of [^{14}C]-Polyaminopropyl Biguanide.[18] In contrast, the results from a study in which groups of Wistar Han rats received [^{14}C]-Polyaminopropyl Biguanide in drinking water, or in the diet for 7 days, indicated that most was absorbed, and that excretion was primarily via the urine.[20]

Toxicological Studies

Acute Toxicity Studies

Animal. The acute dermal, oral, and inhalation toxicity studies in animals summarized below are presented in Table 5.

Dermal. There was no mortality or other signs of systemic toxicity in rats that received a single dermal dosage of 5000 mg/kg aqueous Polyaminopropyl Biguanide (96% pure, in distilled water), but hemorrhage of dermal capillaries at the application site was observed.[2,18] In another acute dermal toxicity study involving rats that received a topical application of 20% aqueous Polyaminopropyl Biguanide, none of the animals died.[21] In an acute dermal toxicity study of 20% aqueous Polyaminopropyl Biguanide involving rabbits, the LD_{50} was reported to be > 400 mg/kg.[18]

Oral. An LD_{50} of 1040 mg/kg was reported in a study in which rats were dosed orally with Polyaminopropyl Biguanide (concentration not stated) in distilled water.[18] LD_{50} values of > 1000 mg/kg were reported for rats dosed orally with aqueous solutions of up to 25% Polyaminopropyl Biguanide.[18,21] A median lethal dosage of 25.6 mg/kg was reported for rats dosed orally with a 0.4% Polyaminopropyl Biguanide solution.[22]

Inhalation. LC_{50} s of > 0.36 mg/L and equal to 0.37 mg/L were reported in acute inhalation toxicity studies in which rats were exposed for 4 hours to Polyaminopropyl Biguanide (99.6%) solutions at concentrations of 360 mg/m^3 in air and up to 300 mg/m^3 in air, respectively.[18] Dark/red lungs were observed at necropsy. A concentration-related depression of respiratory rate was reported in a study in which mice were exposed to Polyaminopropyl Biguanide (20% aqueous) at concentrations up to 208 mg/m^3.[18]

Human

Risk assessment—oral. The EPA conducted a screening-level acute dietary human health risk assessment for Polyaminopropyl Biguanide in food.[14] Risk estimates were calculated for females 13 to 50 years old, the only population subgroup with an acute toxicity end point (not stated) that was of concern. "Risk estimates for the use with the highest exposures were 9% of the acute Population Adjusted Dose (aPAD = 0.2 mg/kg/day) and, therefore, were not of concern." The EPA defines an aPAD as a dose at which an individual could be exposed on any given day and no adverse health effects would be expected.

Short-Term Toxicity Studies

The short-term dermal, oral, and inhalation toxicity studies summarized below are presented in Table 6.

Dermal. No specific systemic effects were observed after 25% aqueous Polyaminopropyl Biguanide was applied to the skin of rats for 3 alternating 24-hour periods.[21] There was also no evidence of systemic toxicity in rats that received 6 24-hour dermal applications of 20% Polyaminopropyl Biguanide (diluted with water to 0.04% active ingredient). There were no mortalities or signs of systemic toxicity in rats that received dermal applications of 20.2% aqueous Polyaminopropyl Biguanide at dosages up to 200 mg/kg daily over a 30-day period (21 applications total; no-observed adverse effect level [NOAEL] = 200 mg/kg/d).[18] In a 21-day dermal toxicity study involving rabbits, there was no evidence of toxic effects on the skin after 20% aqueous Polyaminopropyl Biguanide (12,000 ppm solution [1 mL]) was applied daily.[18]

Oral. Gastrointestinal inflammation was observed in rats dosed orally with 25% aqueous Polyaminopropyl Biguanide (in distilled water; initially at 1 g/kg and subsequently at 0.5 g/kg) for 21 days.[21] A lowest observed adverse effect level (LOAEL) of 0.1 mg/mL for 20% aqueous Polyaminopropyl Biguanide (in

drinking water) was reported in 28-day oral toxicity studies involving rats and mice.[18] Rats (groups of 10) that received Polyaminopropyl Biguanide (in drinking water, up to 150 mg/kg) for 4 weeks experienced dehydration, clinical signs of rough coat and hunched posture, and body weight loss (all classified as severe).[20] Across the 3 dose groups, 10 rats had to be terminated due to severe weight loss, whereas, the remaining rats eventually adapted and began to gain weight. Absolute liver weights in all dose groups were similar to the control group. Mild centrilobular hypertrophy in the liver was observed in some of the rats (all dose groups). In the same study, Polyaminopropyl Biguanide administered (in the diet, 4000 mg/kg) to rats for 4 weeks caused a statistically significant decrease in body weight and absolute liver weight. In this dietary group, there was no evidence of centrilobular hypertrophy in the liver. Also, there was no evidence of necrosis or inflammatory lesions in the liver when Polyaminopropyl Biguanide was administered in drinking water or in the diet. In a 60-day gavage study on Polyaminopropyl Biguanide involving rats, mild toxicity in the liver or kidneys was observed (by microscopic examination) at 2 mg/kg/d (dose equivalent to 0.2 mg/L of 0.4% solution of test substance), 8 mg/kg/d (dose equivalent to 0.4 mg/L of 0.4% solution of test substance), and 32 mg/kg/d (highest dose, equivalent to 1.2 mg/L of 0.4% solution of test substance).[22] None of the animals died.

Inhalation. In 21-day and 28-day inhalation toxicity studies on Polyaminopropyl Biguanide involving rats, no observed adverse effect concentrations (NOAECs) of 0.025 mg/m^3 and 0.0239 mg/m^3 were reported, respectively.[18] In the 21-day study, the animals were exposed (nose-only, concentrations up to 26 mg/m^3) to the test substance 5 days per week, 6 hours/day. Slightly-to-moderately severe pneumonitis was observed at histopathological examination in rats exposed to 0.25 mg/m^3. Moderate to severe pneumonitis was observed in rats exposed to 2.75 mg/m^3, and severe nasal irritation and dyspnea were observed at a concentration of 12.5 mg/m^3. Additionally, all rats of the 12.5 and 26 mg/m^3 groups died. In the 28-day study (nose-only, concentrations up to 2.5 mg/m^3, 6 hours/day, 5 days per week), squamous metaplasia was observed in the larynx of males and females exposed to 0.25 mg/m^3 and 2.5 mg/m^3, and tracheal inflammation was observed in males and females exposed to 2.5 mg/m^3. Pneumonitis and bronchitis were observed in the lungs of males and females exposed to 2.5 mg/m^3.

Subchronic Toxicity Studies

The subchronic oral toxicity studies summarized below are presented in Table 7.

Oral. There were no treatment-related macroscopic postmortem findings in mice in a 90-day drinking water study of 20% aqueous Polyaminopropyl Biguanide (concentrations up to 0.3 mg/mL in drinking water),[3] and a NOAEL of 1000 ppm was reported for this ingredient in a 90-day feeding study on 20.2% aqueous Polyaminopropyl Biguanide in which mice received concentrations up to 4000 ppm in the diet.[18] The following results were reported in 90-day oral toxicity studies on Polyaminopropyl Biguanide involving rats: no mortalities, but iron pigment/deposits were observed in Kupffer cells at 1250 ppm (in one study on 25% aqueous Polyaminopropyl Biguanide) and 5000 ppm (in another study on 25% aqueous Polyaminopropyl Biguanide) in diet; and a NOAEL of 1000 ppm (in a third study on 20.2% aqueous Polyaminopropyl Biguanide).[18,21] An NOAEL of 5500 ppm was reported for Beagle dogs fed Polyaminopropyl Biguanide at concentrations up to 11,000 ppm in the diet for 90 days.[18,21]

Chronic Toxicity Studies

Animal. The chronic dermal and oral toxicity studies summarized below are presented in Table 8.

Dermal. In an 80-week chronic toxicity study involving mice (dermal applications 5 days/week), a mortality rate of 75% was reported for the highest dose group (10% Polyaminopropyl Biguanide; 30 mg dose).[3,21] The exophthalmos observed throughout the study was more severe in this group, compared with the other groups, but the results of histological examination of the eyes and gross and microscopic examination of the thyroids were negative. An NOAEL of 0.6 mg/mouse/day (15 mg/kg/d) was reported.

Oral. Mice were fed 20% Polyaminopropyl Biguanide in the diet (concentrations up to 1000 ppm; feeding 1 week prior to mating and during mating [males and females] and continuation of feeding throughout pregnancy and lactation [females]).[21] The offspring of these mice were also fed 20% Polyaminopropyl Biguanide at concentrations up to 1000 ppm in the diet for 97 weeks. For parents and their offspring, feeding with the test substance did not cause macroscopic changes in the spleen or liver.[18] In a 104-week oral toxicity study involving rats, an NOAEL of 2000 ppm (highest concentration tested in diet) was reported for 20.2% Polyaminopropyl Biguanide.[18] This concentration corresponded to $\sim$ 126 mg/kg/d (in male rats) and 162.3 mg/kg/d (in female rats). *A no* observed effect level (NOEL) of 200 *ppm for histopathologic changes was reported in a 122-week oral toxicity study involving rats fed 20% Polyaminopropyl* Biguanide at concentrations up to 2000 ppm in the diet.[21] Increased adrenal weight was reported for males and females at concentrations of 1000 ppm and 2000 ppm in the diet. In a 26-week feeding study involving dogs, dietary concentrations of 1500 ppm and 4500 ppm 20% Polyaminopropyl Biguanide produced concentration-related hepatotoxicity and nephrosis.[21] An NOAEL of 1500 ppm for 20.2% Polyaminopropyl Biguanide was reported in a 1-year feeding study involving dogs; treatment-related histopathological findings in the liver and kidneys were reported in the high-dose group.[18] In this study, groups of animals were fed test-substance concentrations of 300 ppm, 1500 ppm, and 4500 ppm for up to weeks 11/12. The 4500 ppm concentration was

reduced to 3000 ppm for the remainder of the study because high dose males exhibited unexpected signs of toxicity, including marked reddening/peeling of scrotal skin, loss of appetite, body weight loss, and/or indications of liver impairment in the form of elevated plasma alanine transaminase and/or aspartate transaminase activities.

Human

Risk assessment—dermal. In a chronic oral study, rats received diet containing 0, 200, 600, and 2000 ppm Polyaminopropyl Biguanide (corresponding to about 0, 12.1, 36.3, and 126.1 mg/kg bw/day in males; 0, 14.9, 45.3, and 162.3 mg/kg bw/day in females) for 104 weeks (summarized in Table 8).[18] There were no treatment related clinical signs, ophthalmoscopic findings, or effects on any hematological or urinalysis parameters throughout the study. The pathological examination showed no nonneoplastic and no neoplastic findings at any dose level in either sex. However, at 2000 ppm, slightly raised plasma alkaline phosphatase activity, predominantly in females, and a slightly increased incidence of hepatocyte fat and spongiosis hepatis in males were identified. Though an increased incidence of hemangiosarcoma in females was identified (it gave positive results in the trend test, but not statistically significant in the Fisher Exact Test), a subsequent review conducted by a Pathology Working Group concluded that evidence from this rat study did not support a clear treatment-related effect with respect to vascular tumors. Based on these findings, NOAELs of 36 and 45 mg/kg bw/day were derived for male and female rats, respectively. The SCCS further performed a margin of safety (MOS) calculation by using an NOAEL of 36 mg/kg bw/day on the basis of the following additional assumptions:[3] (1) all cosmetics contain 0.1% Polyaminopropyl Biguanide; (2) the NOAEL is 3.1 mg/kg/d, based on oral absorption of 8.5%; and (3) dermal penetration is 4.09%. Dermal penetration was determined using the results of a dermal penetration study summarized earlier in the report in which 1.56% of applied dose was found in the dermis, 0.03% of the absorbed dose was recovered in the receptor fluid, and one standard deviation (2.5%) was added to the absorbed amount.[3,18] The MOS values are 258 (based on cosmetic exposure estimate) and 227 (based on cosmetic exposure estimate + noncosmetic exposure estimate). Thus, the MOS is lower when additional exposure from noncosmetic use is incorporated.

Risk assessment—inhalation. The Council survey of maximum reported use concentrations by product category provided to CIR on July 18, 2017, indicates that Polyaminopropyl Biguanide is not used in pump or propellant hair sprays.[23] However, products categorized as Tonics, Dressings, and Other Hair Grooming Aids that contain Polyaminopropyl Biguanide at maximum use concentrations of up to 0.1% are reported in the survey, and it is possible that products included in this category are sprays. Furthermore, 2019 FDA VCRP data indicate that Polyaminopropyl Biguanide is used in the Other Fragrance Preparations product category (use concentration data were not provided by industry for this use). Given the potential for inhalation exposure, CIR performed a risk assessment using the ConsExpo Web Spray Model (Consumer Exposure Model, Web version 1.0.1).[8,24-27] The maximum concentrations of use (0.0004% in propellant hair sprays and 0.053% in pump hair sprays) included in this risk assessment to estimate the inhalation exposure concentrations of Polyaminopropyl Biguanide during the use of cosmetic spray products are based on survey results that were originally submitted by the Council to CIR on April 11, 2017.[6]

The parameters used in this risk assessment are presented in Table 9. Conservative default values published by Rijksinstituut voor Volksgezondheid en Milieu (RIVM—the Dutch National Institute for Health and Environment) were used in all of the calculations.[8] One exception is that the room ventilation rate was assumed to be 0.2 room-air exchanges per hour, which is the default value specified in European Union's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) guidance, rather than the 2 exchanges per hour indicated by RIVM guidance for bathrooms.[25] The more conservative value (0.2/h) appears to be more appropriate to represent low-end air-exchange rates in homes in the United States, in which ventilation fans may not be used routinely. No default values were available specifically for pump hair spray products. Thus, the spray duration assumed for propellant hair sprays (14.4 s) and default values for pump toilet-water sprays were used in the calculations for pump hair sprays.

The use of conservative default values for multiple exposure parameters ensures that high-end, "reasonable worst-case" exposures are calculated.[8,25] Generally, the exposure concentrations predicted by the ConsExpo Model increase with increasing spray durations and decrease with increasing exposure durations/event (ie, the time over which the exposure concentrations are averaged after each spraying event).

The average Polyaminopropyl Biguanide inhalation exposure concentrations over the 5-minute default exposure duration/event were 0.00012 mg/m^3 for propellant hair sprays and 0.0022 mg/m^3 for pump hair sprays.

The NOAEC was approximately 0.024 mg/m^3 in a 28-day inhalation study in which rats were exposed, nose only, to Polyaminopropyl Biguanide in an aerosolized water solution, 6 h/day, 5 days/week.[3] Margin of safety values were calculated by dividing the NOAEC by the average inhalation exposure concentrations/event estimated using the ConsExpo model. The MOS values were 200 for propellant hair sprays and 11 for pump hair sprays.

An MOS of 100 may be considered to be adequate to allow for the uncertainties associated with using the NOAEC from a short-term rat study to evaluate potential chronic human exposures (ie, 10 for short-term to long-term exposure extrapolation $\times$ 10 for interspecies extrapolation = 100). Accordingly, the ConsExpo Web model was used to calculate concentrations of use that would yield an MOS of 100 for Polyaminopropyl Biguanide in pump and propellant hair spray products and propellant deodorant products. The results indicate that use concentrations of 0.0058% in pump hair sprays, 0.00084% in

propellant hair sprays, and 0.000055% in propellant deodorant sprays would each be associated with an MOS of 100.

The daily exposure duration in the rat study (6 hours) from which the NOAEC was derived (ie, 6 h/day or 360 min/day) is 72 times greater than the exposure duration of a person using a hair spray once a day (1 event/day × 5 min/event = 5 min/day), 5 days per week, and 24 times greater than the exposure duration of a person using a hair spray 3 times a day 5 days/week.

The daily exposure duration in the rat study is about 7 times greater than the exposure duration would be for a beautician applying hair spray to customers, an average of 10 times a day, 5 days/week. The beautician's occupational exposure may be reduced by workplace ventilation systems and larger room volumes, as well as the direction of the spraying (ie, away from the beautician).

Risk assessment—various exposure routes. The US EPA assessed the human health risks associated with residential-handler and post-application pesticide exposure scenarios (including pesticides containing Polyaminopropyl Biguanide) using surrogate exposure data, maximum application rates (specified on the product labels), and standard assumptions.[14] Residential handler exposures may occur when individuals mix, load, or apply a pesticide. Post-application exposures may occur to bystanders affected during or after application by others. The agency determined that all margins of exposure (MOEs) from dermal and inhalation exposure for residential handlers are above the protective target of at least 100 and, therefore, were not concerning. For post-application dermal and incidental ingestion (oral exposures) scenarios, MOEs calculated based on an oral NOAEL of 20 mg/kg/d were also above the EPA's level of concern.

Chronic dietary risk estimates were provided for the general US population and all population subgroups.[14] The chronic Population Adjusted Dose (cPAD) is the level of exposure (mg/kg/d) that the EPA determines should not be exceeded. These estimates were below EPA's level of concern for the general US population (ie, < 10% of the cPAD) and all population subgroups (ie, < 37% of the cPAD for children). Therefore, the chronic dietary risk is not of concern.

1The aggregate risk assessment integrates the assessments that were conducted for dietary and residential exposure. Aggregate calculations were performed for adults and children using the Aggregate Risk Index (ARI) method. Aggregate Risk Index values were greater than 1.2 for children and greater than 5.4 for adults, and these risks were determined not to warrant the EPA's concern.[14] As a general rule, an ARI of ≥ 1 is of little concern, but an ARI of < 1 suggests a risk that is of concern.

Developmental and Reproductive Toxicity Studies

The developmental and reproductive toxicity studies summarized below are presented in Table 10.

An NOAEL of 10 mg/kg/d for developmental toxicity was reported in an oral dosing (by gavage on gestation days 6 through 15) study on 20% aqueous Polyaminopropyl Biguanide involving mice.[18,28] In oral reproductive and developmental toxicity studies on 20% aqueous Polyaminopropyl Biguanide in rats, NOAECs of 1000 ppm (after feeding in diet on gestation days 1 through 20)[18] and 1300 ppm (after feeding in diet during a 9-day premating period and until the 3rd generation)[18,21] have been reported. An NOAEL of 2000 ppm for reproductive and development effects was reported in a study in which rats were fed (through 2 successive generations) with 20.2% aqueous Polyaminopropyl Biguanide at dietary concentrations up to 2000 ppm.[18] Polyaminopropyl Biguanide has been classified as embryotoxic at a dosage rate of 100 mg/kg/d (rats; protocol not stated).[29] An NOAEL of 40 mg/kg/d for developmental toxicity has been reported in an oral dosing (20.2% aqueous Polyaminopropyl Biguanide by gavage on gestation days 8 through 20) study involving rabbits.[18] Polyaminopropyl Biguanide (0.04% in polyethylene glycol [PEG]) has been classified as embryotoxic in rabbits at an oral dosage rate of 32 mg/kg/d (animal strain and dosing protocol not stated).[29] Polyaminopropyl Biguanide (concentration not stated) has been classified as teratogenic in rats at an intraperitoneal dosage rate of 10 mg/kg/d (dosing protocol not stated).[29] In an inhalation study on 20% aqueous Polyaminopropyl Biguanide, degeneration of seminiferous tubules in the testis of 1 male rat was observed after exposure to 0.25 mg/m^3 (6 h/day, 5 days/week for 3 weeks), but this was not observed in any other group, including the group exposed to the highest concentration (26 mg/m^3).[18]

Genotoxicity Studies

The genotoxicity studies (in vitro and in vivo) summarized below are presented in Table 11.

In Ames tests, ~20% Polyaminopropyl Biguanide was nongenotoxic at doses up to 5000 μg/plate, with and without metabolic activation.[18] At the highest dose evaluated (333,300 μg/plate) in the Ames test, Polyaminopropyl Biguanide was weakly genotoxic in *Salmonella typhimurium* strain TA 1538 without metabolic activation. Polyaminopropyl Biguanide was nongenotoxic in a mouse lymphoma assays at concentrations up to 2000 μg/mL with and without metabolic activation, or in an in vitro micronucleus test (cultured human peripheral blood lymphocytes) at concentrations up to 50 μg/mL (without metabolic activation) and up to 250 μg/mL (with metabolic activation). In an in vivo micronucleus test, Polyaminopropyl Biguanide was nonclastogenic in polychromatic erythrocytes from mice that received single oral dosages up to 400 mg/kg. In an in vivo unscheduled DNA synthesis assay, there was no induction of unscheduled DNA synthesis in hepatocytes from rats that received single oral doses up to 1500 mg/kg.

Carcinogenicity Studies

The carcinogenicity studies (in vitro, dermal, and oral) summarized below are presented in Table 12.

In Vitro

Polyaminopropyl Biguanide (20%) was evaluated at concentrations up to 3000 μg/mL in the cell transformation assay (using baby hamster kidney fibroblasts); there was no difference in the number of transformed cell colonies between test and negative control cultures.[18] In another assay, RAW 264.7 mouse macrophages (a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice) were cocultured with SVEC-10 mouse liver endothelial cells in various experimental conditions: preactivation of macrophages with Polyaminopropyl Biguanide or lipopolysaccharide (LPS) and/or coculture in presence of Polyaminopropyl Biguanide.[3] Polyaminopropyl Biguanide, tested at concentrations up to 1 ppm, had no direct effect on liver cell proliferation and did not potentiate cell proliferation induced by activated macrophages.

Animal

Dermal. Polyaminopropyl Biguanide was classified as a hepatocarcinogen in mice at the highest dose tested in a study in which Polyaminopropyl Biguanide in ethanol was applied to the skin daily at doses up to 750 mg/kg/d (5 days/week) for 80 weeks.[21] The NOAEL was 0.6 mg/mouse/day (15 mg/kg/d). A variety of inflammatory hepatic changes was observed in all groups, including the controls. However, at 750 mg/kg/d, severe hepatitis was observed in some of the animals. These hepatic changes appeared to have been mainly responsible for causing increased numbers of deaths in the high-dose group. (Additional study results are included in the 80-week chronic dermal toxicity study that is summarized earlier in this safety assessment.) A scientific advisory panel advising the SCCS indicated that the hepatitis observed in this study may be attributable to the *Helicobacter hepaticus* infections, which may also be responsible for the increased incidence of hepatocellular neoplasms in these animals.

Oral. Mice were fed 20.2% Polyaminopropyl Biguanide in the diet (up to 1000 ppm active ingredient; feeding 1 week prior to mating and during mating [males and females]).[18] The offspring of these mice were also fed 20.2% Polyaminopropyl Biguanide at concentrations up to 1000 ppm active ingredient in the diet for 97 weeks. Except for vascular tumors, there were no treatment-related (nonneoplastic or neoplastic) increases in histopathologic findings. Vascular tumors (hemangiosarcomas or hemangiomas) in the liver or other sites and a high mortality incidence (80%) were reported by week 97. A dose-related increase in the liver vascular tumor incidence was reported. After reviewing the results of this study, the SCCS concluded that the data are considered to be of low reliability due to the high mortality rate that was reported. When mice were fed Polyaminopropyl Biguanide at dietary concentrations up to 4000 ppm for up to 28 days, increased cell proliferation in a concentration-related manner was noted at 1200 ppm and 4000 ppm.[3] In another study, a statistically significant increase in the incidence of hemangiosarcomas and hemangiomas was reported in male mice (C57B1/10J/CD-1 strain) that received Polyaminopropyl Biguanide at a dietary concentration of 4000 ppm daily for 2 years.[18] A low incidence of hemangioma (2 of 64 males; 2 of 64 females) and hemangiosarcoma (1 of 64 females) was reported in a study in which rats were fed Polyaminopropyl Biguanide at a dietary concentration of 2000 ppm for 2 years.[30] In another 2-year study, Polyaminopropyl Biguanide was administered (in drinking water or in diet) to groups of rats. [20] Hepatocellular tumors were induced at concentrations (in water) of 1000 mg/L and 1500 mg/L, but not at a concentration of 500 mg/L (in water). Administration of Polyaminopropyl Biguanide in diet did not cause increase in hepatocellular tumors. The hypothesized mode of action (MOA) for liver tumors induced by Polyaminopropyl Biguanide in drinking water involves increased hepatocyte proliferation and induction of hepatocellular foci and tumors.[20] Polyaminopropyl Biguanide was classified as noncarcinogenic in rats fed dietary concentrations up to 2000 ppm for 122 weeks; mortality was 80%.[21] In a feeding study in which rats were fed Polyaminopropyl Biguanide at concentrations up to 2000 ppm, 80% mortality was reported at 124 weeks.[18] A low incidence of hemangiomas or hemangiosarcomas (mostly in lymph nodes) was observed in the groups of remaining animals (7 groups, with 8 to 21 rats/group; 1 animal with a hemangioma or hemangiosarcoma per group).

Other Relevant Studies

Effect on Lung Cells

A study was performed to characterize the inflammatory responses, including the mechanism of action, induced in lung cells exposed to Polyaminopropyl Biguanide.[31] A549 cells that were exposed to Polyaminopropyl Biguanide showed concentration-dependent (0 to 80 μg/mL) decreased viability, significant reactive oxygen species (ROS) generation (at 20 μg/mL), inflammatory cytokine secretion (statistically significant increase in tumor necrosis factor alpha [TNF-α] release at 20 μg/mL), and nuclear factor kappa B (NF-κB) activation (expression of IκB-α protein significantly degraded at concentrations $>$ 10 μg/mL). Statistically significant cytotoxicity to A549 cells was observed at concentrations $>$10 μg/mL. Polyaminopropyl Biguanide triggered inflammatory cytokine secretion and NF-κB activation by modulating the degradation of IκB-α and through the accumulation of nuclear p65. It was noted that TNF-α plays important roles in interleukin 8 (IL-8) expression as well as in NF-κB activation. Interleukin 8 production induced by Polyaminopropyl Biguanide was completely suppressed by an NF-κB inhibitor, but not by an ROS scavenger. The authors suggested that Polyaminopropyl Biguanide induces inflammatory responses via the NF-κB signaling pathway.

Other Cellular Effects and Antimicrobial Activity

Polyaminopropyl Biguanide (PHMB; C6) was compared to the (structurally) closely related polyaminopropyl biguanide (C3)

with respect to antiseptic efficacy and cytotoxicity in vitro.[32] Antimicrobial efficacy tests were performed via determination of the minimum bactericidal concentration. Polyaminopropyl Biguanide (PHMB; C6) exhibited high antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* (minimal bactericidal concentration $\leq$ 0.05 mg/mL [0.005%]), whereas polyaminopropyl biguanide (C3) proved to be ineffective in bacterial eradication. These results suggest that even small differences in the chemical structure of related agents, such as Polyaminopropyl Biguanide (PHMB; C6) and polyaminopropyl biguanide (C3), can substantially affect their efficacy.

Cytotoxicity was evaluated in human keratinocytes (HaCaTs) and murine fibroblasts (L929).[32] In fibroblast or keratinocyte cultures, concentrations for both test substances ranged from 0.005% to 1% vol/vol. Polyaminopropyl biguanide (C3) was also tested at concentrations ranging from 0.25% to 3% vol/vol. Cultures were incubated for up to 72 hours. For all tested concentrations, Polyaminopropyl Biguanide (PHMB; C6) was highly cytotoxic to human HaCaT and L929 murine fibroblast cell after 24 and 72 hours of incubation, never exceeding a survival rate of 27%. Polyaminopropyl biguanide (C3) displayed significantly lower cytotoxicity at concentrations ranging from 0.005% to 0.1% vol/vol. At concentrations up to 0.1%, no cytotoxic effect could be detected in L929 cells after 24 hours, whereas, for HaCaT cells, moderate and high cytotoxicity was evident at 0.05% and 0.1% polyaminopropyl biguanide (C3). After 72 hours, only a weak cytotoxic effect on L929 cell at 0.05% and 0.1% polyaminopropyl biguanide (C3) could be observed, while, for HaCaT cells, concentrations up to 0.1% were classified as noncytotoxic. However, concentrations $\geq$ 0.25% polyaminopropyl biguanide (C3) were highly cytotoxic to cells of both cell lines after 24 hours of incubation. When compared directly, polyaminopropyl biguanide (C3) consistently resulted in a significantly higher cell survival rate than Polyaminopropyl Biguanide (PHMB; C6), irrespective of concentration and incubation time ($P \leq 0.0006$).

It has been hypothesized that exposures to Polyaminopropyl Biguanide may have epigenetic effects, including nongenotoxic DNA base modifications (eg, changes in DNA-base methylation) and altered mitogenic cytokine production.[33] These effects have been assessed *in vitro* using 3 cell types: Caco-2 cells (from a human colon adenocarcinoma) with a p53 nonfunctional gene (Δp53: mut p53), Neuro-2A (mouse neuroblastoma cells), and HepG2 cells (human hepatocellular carcinoma) with functional p53 genes. The studies focused mainly on Polyaminopropyl Biguanide effects on the liver, but also examined the gut and brain since these are also the target organs. At Polyaminopropyl Biguanide concentrations of 1μg/mL to 20 μg/mL, neither a growth stimulatory effect nor a growth inhibitory effect was observed. Viability testing using neutral red resulted in an IC_{50} of 20 to 25 μg/mL after treatment with Polyaminopropyl Biguanide for 3 hours, whereas the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability test led to IC_{50} of 80 μg/mL, 160 μg/mL, and 160 μg/mL for HepG2 cells, Neuro-2A cells, and Caco-2 cells, respectively. The neutral red test showed that the cell lines had similar sensitivity to Polyaminopropyl Biguanide at much lower concentrations than with the MTT assay, indicating that the cellular target is the membrane. (The principle of neutral red test is based on the integrity of the cellular, lysosomal and endosomal membrane, while the MTT test is an indicator of metabolic activity in living cells.) Polyaminopropyl Biguanide does not induce significant oxidative stress (as determined by measuring production of malondialdehyde or lipoperoxidation, nor does it induce hydroxylation of DNA (8-hydroxy-2′-deoxyguanosine) and/or its hypermethylation (5-methylcytosine [m5dC] content), the latter being strongly implicated in DNA replication and regulation and cell division.

Additional results from this study indicated that Polyaminopropyl Biguanide did not induce significant production of mitogenic cytokines, such as TNF-α (tumor necrosis factor-alpha), interleukins (IL-1 alpha), and NF-κB, which can cause either apoptosis or stimulate the growth of transformed cells or tumors. Instead, concentrations of 20 to 100 μg/mL Polyaminopropyl Biguanide killed cells of all types in less than 3 hours. The expression of genes involved in the mechanisms of cell death induced by Polyaminopropyl Biguanide, including p53, the pro apoptotic gene bax and others, and the anti-apoptotic bcl-2 and caspase-3 genes, has been evaluated using reverse transcription polymerase chain reaction methodology. Results indicated that it does not appear that Polyaminopropyl Biguanide-induced cell death is the result of apoptosis, but, rather, is cytotoxic at the cell membrane level, resulting in necrotic cell death. Finally, there was no apparent inhibition of GAP-junctions (ie, gap junctional intercellular communication) in the presence of Polyaminopropyl Biguanide. Taken together, the data indicate that Polyaminopropyl Biguanide did not exhibit clear or remarkable epigenetic effects, except for a slight increase in the levels of some cytokines and a transcription factor at concentrations that cause rapid cell lysis.

Dermal Irritation and Sensitization Studies

The skin irritation, sensitization, and phototoxicity/photosensitization studies summarized below are presented in Table 13.

Irritation

In a study involving mice, the highest dose of Polyaminopropyl Biguanide (10% concentration in ethanol, 30 mg dose) caused hyperkeratosis and, occasionally, ulceration extending into the dermis when applied repeatedly for 80 weeks.[21] Polyaminopropyl Biguanide (0.04%) was classified as a nonirritant when applied to the skin of rats for 24 hours. Repeated applications of 20.2% aqueous Polyaminopropyl Biguanide to rats for 21 days resulted in slight skin irritation (at 60 mg/kg/d) and moderate irritation (at 200 mg/kg/d).[18] Severe skin irritation was observed in all rats that received a single 24-hour application of 25% aqueous Polyaminopropyl Biguanide at dosages of 2.5 mL/kg and 5 mL/kg.[21] Focal ulceration and edema were observed in rats after 25% aqueous Polyaminopropyl Biguanide was applied repeatedly to the skin. Repeated 23-hour

applications of Polyaminopropyl Biguanide (12,000 ppm; 1 mL per application) to the skin of rabbits for 21 days were not irritating. Single applications (24 hours) of 20% aqueous Polyaminopropyl Biguanide to rabbits indicates that this compound is noncorrosive, but moderately irritating, to intact skin, and severely irritating to abraded skin.[18] Polyaminopropyl Biguanide (20% aqueous) induced erythema at abraded, but not intact, skin sites in rabbits, and was classified as a noncorrosive material. Moderate erythema was observed after 20% aqueous Polyaminopropyl Biguanide was applied for 24 hours to the skin of rabbits. Mild skin irritation resulted after Polyaminopropyl Biguanide (96%, as powder) was applied to the skin of rabbits for up to 4 hours.[3] Polyaminopropyl Biguanide (0.5 g, moistened with water; single 4-hour application) was also classified as a mild skin irritant in rabbits.[18] Slight to moderate erythema was observed in guinea pigs that received repeated applications of 25% aqueous Polyaminopropyl Biguanide for 3 days.[18]

Polyaminopropyl Biguanide (up to 1.5% active) was not classified as a primary skin irritant when applied for 24 hours to the skin of human subjects (17 males and 28 females).[18] A bacterial nonocclusive dressing loaded with 1% wt/vol sericin and 0.3% wt/vol Polyaminopropyl Biguanide was classified as nonirritating after application to the skin of 105 subjects for 3 days.[34]

Sensitization

Results of a local lymph node assay (LLNA) were positive with Polyaminopropyl Biguanide.[35,36] In maximization tests on Polyaminopropyl Biguanide, moderate skin sensitization was observed in guinea pigs induced with 0.06% active ingredient (intradermal injection) and 20.2% active ingredient (occlusive application) and challenged with Polyaminopropyl Biguanide (20.2% active ingredient) and a 30% solution of the ingredient (6% active ingredient) in deionized water, and moderate to strong sensitization was observed in guinea pigs induced with 0.2% active ingredient (intradermal injection) and 20.2% active ingredient (topical application) and challenged with Polyaminopropyl Biguanide (20.2% active ingredient).[18] In another guinea pig maximization test, sensitization was not observed in guinea pigs induced with 0.15% (intradermal injection) and 20% Polyaminopropyl Biguanide (topical application) and challenged with 10% or 20% Polyaminopropyl Biguanide. In one Buehler test on Polyaminopropyl Biguanide, guinea pigs were induced with 2% active ingredient (topical application), challenged with 2% active ingredient, and rechallenged with 0.2%, 2%, and 4% active ingredient. The initial challenge with 2% active ingredient and rechallenge with 2% and 4% active ingredient resulted in faint erythema; rechallenge with 0.2% active ingredient produced negative results. Polyaminopropyl Biguanide (2% active ingredient) was classified as a moderate sensitizer. In another Buehler test, it was determined that the threshold for eliciting sensitization in guinea pigs was $\sim$ 1%. Induction concentrations ranged from 0.3% to 5% and challenge concentrations ranged from 0.075% to 15%. Results from a study evaluating the possible cross-reactivity of Polyaminopropyl Biguanide (challenge with 20%) with chlorhexidine (challenge with up to 4% chlorhexidine gluconate) in guinea pigs were negative.

In a human repeated insult patch test (HRIPT; 191 subjects), it was determined that 20% aqueous Polyaminopropyl Biguanide (2% active ingredient; effective concentration = 0.4%) was not capable of causing primary skin irritation, but was capable of causing sensitization.[3] When a leave-on product containing 20% Polyaminopropyl Biguanide (tested at 0.5%, effective concentration 0.1% Polyaminopropyl Biguanide) was evaluated in an HRIPT involving 207 subjects, it was concluded that the product did not induce dermal sensitization.[37] In another HRIPT (115 subjects; any ethnicity, provided that their degree of skin pigmentation did not significantly interfere with evaluations) on a neck cream containing 0.2% Polyaminopropyl Biguanide, the product did not cause clinically meaningful irritation or sensitization.[38] In another study, the skin sensitization potential of 20% Polyaminopropyl Biguanide (diluted with distilled water to 1% vol/vol prior to testing; 750 $\mu g/cm^2$) was evaluated using semi-occlusive patches in an HRIPT involving 108 subjects (Asian [$\sim$2%], Biracial [$\sim$3%], Black [$\sim$ 23%], Caucasian [$\sim$ 33%], and Hispanic [$\sim$39%]; Fitzpatrick skin types not stated).[39] The authors concluded that Polyaminopropyl Biguanide did not induce dermal sensitization in the subjects tested, and a no expected sensitization induction level (NESIL, determined based on data from this HRIPT) of 750 $\mu g/cm^2$ was used to calculate quantitative risk assessments (QRA) on various product types.

Risk Assessment

A series of QRAs was performed by industry in response to the Panel's concerns about sensitization potential.[40] The QRA for contact dermatitis with Polyaminopropyl Biguanide in cosmetics utilized a NESIL of 1000 $\mu g/cm^2$, which supports the use of this ingredient at concentrations of $\leq$ 0.1%. Among the human data that were used to derive the NESIL was a phototoxicity/photosensitization study (described below) involving 26 subjects tested with 1% Polyaminopropyl Biguanide at a dose of 1000 $\mu g/cm^2$, the highest nonsensitizing dose in relation to all of the HRIPT data that were considered. The NESIL of 1000 $\mu g/cm^2$ was used to determine whether estimated exposure, using maximum use concentrations from a Council survey, could be considered safe. The ratio of AEL (Acceptable Exposure Level)/CEL (Consumer Exposure Level) was $>$ 1, except for the product that contained 0.2% Polyaminopropyl Biguanide (Table 14).

A second QRA, performed by industry, utilized a NESIL of 750 $\mu g/cm^2$. The human data that were used to derive the NESIL are from an HRIPT involving 108 subjects (Asian [$\sim$2%], Bi-racial [$\sim$3%], Black [$\sim$ 23%], Caucasian [$\sim$ 33%], and Hispanic [$\sim$39%]) tested with 1% vol/vol Polyaminopropyl Biguanide, that is summarized earlier in this report.[39] This QRA resulted in similar ratios of AEL/CEL values $>$1, and the same exception for the product that contained 0.2% Polyaminopropyl Biguanide (Table 15).[41]

Photosensitization/Phototoxicity

Animal. The photoirritation potential of 20% aqueous Polyaminopropyl Biguanide was studied using 10 male rats.[21] The following two concentrations of the test substance (in distilled water) were evaluated: 10% (effective concentration = 2%) and 25% (effective concentration = 5%). Each test concentration (0.1 mL) was applied to dorsal skin once daily for 4 days. The test site was irradiated with short-wave ultraviolet light (UVC; black lamp) for 3 hours daily. Very strong irritation potential, but no significant photoirritancy, was reported in a study in which male rats were tested with Polyaminopropyl Biguanide at concentrations of 2% and 5%.

Human. A phototoxicity/photoallergenicity study on 20% aqueous Polyaminopropyl Biguanide was performed using 26 male and female subjects.[21] The test substance was diluted (1:20 in water; effective concentration = 1%) prior to application. Patches (20 mm × 20 mm Webril pad affixed to a 40 mm × 40 mm adhesive square) were moistened with 0.4 mL of the test substance (dose = 1 mg/cm^2)[42] and applied to the upper arm for 24 hours, 3 times per week for 4 successive weeks. Immediately after patch removal, the sites were exposed to direct rays of mid-day sun for 1 hour. The challenge application occurred at week 6. Polyaminopropyl Biguanide was essentially nonirritating and did not induce sensitization, phototoxicity, or photoallergenicity.

Ocular Irritation Studies

The ocular irritation studies summarized below are presented in Table 16.

Undiluted Polyaminopropyl Biguanide was a severe ocular irritant/corrosive agent when instilled into rabbit eyes.[3] The instillation of 25% aqueous Polyaminopropyl Biguanide into the eyes of rabbits resulted in severe inflammation and corneal damage in unrinsed eyes and slight inflammation in rinsed eyes.[21] Moderate and mild ocular irritation were observed in unrinsed and rinsed rabbit eyes, respectively, after 20% aqueous Polyaminopropyl Biguanide was instilled.[3] In another study involving rabbits, the instillation of Polyaminopropyl Biguanide (25% aqueous) into the eyes induced slight inflammation, but no corneal ulceration.[21] Ocular irritation was not observed when Polyaminopropyl Biguanide (0.04% active ingredient) was instilled into the eyes of rabbits.[21] In a study in which 20% aqueous Polyaminopropyl Biguanide (100 µL) was instilled into human eyes (from cadavers) and the eyes of rabbits in a temperature-controlled chamber (32 °C-36 °C), normal corneal morphology was observed at histological examination.[43]

Clinical Studies

The patient multicenter studies summarized below are presented in the Human Sensitization Studies section of Table 13. In another type of clinical study, no adverse effects were noted following the exposure of 29 patients to a preoperative antiseptic for cataract surgery that contained 0.2% Polyaminopropyl Biguanide.[44]

Retrospective and Multicenter Studies

In a multicenter study involving 374 patients (United Kingdom study) patch tested with 2.5% aqueous Polyaminopropyl Biguanide, 2 sensitization reactions were reported.[45,46] In a second multicenter study (German study) involving 1554 patients, sensitization reactions were observed in 6 patients patch tested with 0.5% Polyaminopropyl Biguanide.[47] It was noted that this initial series of data suggested that the baseline frequency of Polyaminopropyl Biguanide sensitization was very low (0.5% and 0.4% in the United Kingdom and German studies, respectively). The majority of positive reactions were considered weak. It was noted that these data suggested that Polyaminopropyl Biguanide may not be a relevant contact allergen.

In a subsequent German multicenter study involving 1975 patients, 10 patients had sensitization reactions to 0.5% Polyaminopropyl Biguanide (20% aqueous Polyaminopropyl Biguanide tested at 2.5% concentration) and 16 patients had sensitization reactions to 1% Polyaminopropyl Biguanide.[48] The majority of the positive reactions were considered weak. When results of the 3 studies were considered together, it was noted that the frequency of sensitization reactions to Polyaminopropyl Biguanide remained low and stable, in spite of the use of Polyaminopropyl Biguanide in underarm deodorants.

Contact Urticaria

A female patient experienced grade III anaphylaxis (immunoglobulin E [IgE]-mediated mechanism confirmed) with palmar pruritus, flush, swelling of lips, swallowing difficulties, hypotension, and loss of consciousness while using a new brand of wet toilet paper containing Polyaminopropyl Biguanide as a disinfectant.[17,49] The detailed allergy history of the patient indicated 3 prior anaphylactic episodes (grade II) during wound care of a leg ulcer. One of the episodes occurred after the use of a wound dressing that contained Polyaminopropyl Biguanide. The other 2 episodes occurred after wound cleansing with 2 different Polyaminopropyl Biguanide disinfectants, one of which contained Polyaminopropyl Biguanide, PEG 4000, and no other additives. The composition of the other disinfectant that contained Polyaminopropyl Biguanide was not detailed. However, according to another publication, the composition of that disinfectant (liquid and gel) is as follows: 0.1% Polyaminopropyl Biguanide, 0.1% undecylenamidopropyl betaine, and water; the gel also contains glycerol and hydroxyethyl cellulose.[50] The patient had no known allergies or atopic diseases. Skin prick tests were positive for the disinfectant of known composition, which was tested in a 1:10 dilution, corresponding to 20 µg/mL Polyaminopropyl Biguanide. Positive skin prick test results were also reported for chlorhexidine in different commercial preparations. Skin prick test results for PEG 4000 were negative, and the same was true for the 5

healthy volunteers who were prick tested with the disinfectant of known composition. Whether or not the other disinfectant containing Polyaminopropyl Biguanide was evaluated in prick tests was not mentioned. Other results reported in this case report indicated that there was limited in vitro cross-reactivity between Polyaminopropyl Biguanide and chlorhexidine. The author noted that patients with known chlorhexidine allergy could be at risk for anaphylactic reactions to Polyaminopropyl Biguanide.

A male patient (atopic and diabetic) had a history of angioedema and pruritus after using wet wipes.[13] Patch test results for an ingredient of the wipes, Polyaminopropyl Biguanide (tested at 1:10 in water), and the wipe itself were negative. However, prick tests resulted in strong positive reactions to the wipe and this ingredient after 15 minutes, and the reactions continued to increase in intensity during the following 2 hours.

The prick test (protocol and test concentration not specified) was used to diagnose immediate contact urticarial reactions in 44 patients with eczematous dermatitis. A positive reaction to Polyaminopropyl Biguanide was observed in 1 patient.[51]

Two cases of severe anaphylaxis were reported following contact of a surgical wound with a hospital disinfectant containing 0.2% Polyaminopropyl Biguanide.[52] Immediate-type hypersensitivity to Polyaminopropyl Biguanide was suggested by positive skin prick tests in both patients and by negative skin tests in control individuals. Skin tests involving chlorhexidine were negative.

The case of a 77-year-old female patient who suffered from severe anaphylaxis during wound (leg ulcer) care was presented.[53] The results of an allergologic evaluation indicated specific IgE antibodies to chlorhexidine (a biguanide antiseptic), but anaphylaxis to chlorhexidine was not congruent with the patient history and dermal provocation tests. However, skin prick tests were indicative of sensitization to Polyaminopropyl Biguanide. These results were supported by the detection of specific IgE antibodies to Polyaminopropyl Biguanide, the results of basophil activation tests, and IgE inhibition analysis. In an assay to assess cross-reactivity, varying concentrations of Polyaminopropyl Biguanide and chlorhexidine (0.1 to 100 μg/mL) were added to the patient's serum. The results of this assay suggested a cross-reaction between Polyaminopropyl Biguanide and chlorhexidine. The authors presumed cross-reactive IgE antibodies binding to both biguanide antiseptics and identified Polyaminopropyl Biguanide as the likely cause of the anaphylactic reaction. Polyaminopropyl Biguanide was recognized as an emerging allergen that has to be considered as a cause of anaphylaxis.

Case Reports

An itchy rash on the hand was observed over a 2-year period in a nonatopic patient with a history of retinal detachment surgery.[54] The patient had regularly used a rinse-off contact lens cleaning solution containing 0.001% Polyaminopropyl Biguanide twice daily. A patch test chamber containing the undiluted contact lens cleaning solution was applied to the skin for 2 days, and doubtful results were reported on day 4. A patch test chamber containing a 10% dilution of the product (0.0001% Polyaminopropyl Biguanide tested) was subsequently applied to the skin, and positive results (+ reaction) were observed on day 7. Additionally, semi-open tests of the undiluted product yielded a weak positive reaction on day 7. In other tests, the individual ingredients (obtained from the manufacturer) of the contact lens cleaning solution were diluted to different concentrations in water. There were no reactions to 2% aqueous Polyaminopropyl Biguanide, but a weak, late reaction (1+ reaction) to 5% aqueous Polyaminopropyl Biguanide was observed on day 7. However, stronger and earlier reactions were observed after the application of 10% aqueous Polyaminopropyl Biguanide (+? reaction on day 2; 2+ reaction on days 5 and 7) and 20% aqueous Polyaminopropyl Biguanide (2+ reaction on day 2; 3+ reaction on days 5 and 7). Patch test results for 20% aqueous Polyaminopropyl Biguanide in 10 control subjects were negative.

In a case report on a nonatopic patient with a history of bilateral leg ulcers and multiple contact allergies, mild hand dermatitis was observed after repeated use of a wound irrigation solution that contained Polyaminopropyl Biguanide and a wound gel containing the same disinfectant.[50] The composition of the disinfectant (liquid and gel) was as follows: 0.1% Polyaminopropyl Biguanide, 0.1% undecylenamidopropyl betaine, and water; the gel also contained glycerol and hydroxyethyl cellulose. In a repeated open application test, a positive reaction was observed after the gel was applied twice daily (in elbow fold) for 10 days. The patient was also patch tested (patch test chamber) with 5% aqueous Polyaminopropyl Biguanide (a dilution of a 20% aqueous solution). The solution was applied to the upper arm for 2 days; reactions, scored according to International Contact Dermatitis Research Group (ICDRG) guidelines were negative on day 2, but were positive on day 4. The patch test (same procedure) was repeated at concentrations of 2.5% and 5% aqueous Polyaminopropyl Biguanide. Positive reactions to the 5% concentration were observed on day 2 (+) and day 4 (++, with partially pustular morphology). Results for the gel and liquid were negative in patch tests.

A chronic, recurrent and itchy dermatitis was observed in a male patient who used wet wipes.[55] Polyaminopropyl Biguanide, an ingredient of the product, was tested at different concentrations (20%, 2%, and 0.2% aqueous). Scoring was performed in accordance with ICDRG guidelines. On day 2 and day 4, respectively, + and ++ reactions to 20% Polyaminopropyl Biguanide (with a papulovesicular reaction, extending outside of the test chamber) were observed; +? and + reactions to 2% Polyaminopropyl Biguanide were observed on days 2 and 4, respectively. No reactions to 0.2% Polyaminopropyl Biguanide were observed.

A nonatopic patient with a history of Crohn disease presented with a dermatitis eruption in the area around where the gastrostomy tube had been inserted. Polyaminopropyl Biguanide was a component of the antimicrobial foam dressing that was used. The patient was patch tested with Polyaminopropyl Biguanide (5% aqueous) at 2 separate sites on the upper back.

At 96 hours, + reactions were observed at both sites. Negative results were reported for the 10 control subjects who were patch tested.[56]

Other Clinical Reports

Based on medical surveillance information obtained between 2004 and 2007 on employees who came in contact with Polyaminopropyl Biguanide in the workplace, no cases of skin sensitization to this chemical were reported.[18] All manufacturing and laboratory employees were offered complete medical evaluations on a regular basis depending on their age. These were conducted every 1 to 2 years.

In a clinical trial (106 dialysis patients) in which patients were treated for infections, Polyaminopropyl Biguanide was well-tolerated and there were only 2 cases of transient local skin erythema.[57] Four of 28 patients were excluded from a cohort study because of adverse effects related to a Polyaminopropyl Biguanide dressing.[58] Reportedly, the application of very high doses (doses not stated) of Polyaminopropyl Biguanide can trigger fever and a generalized exanthema.[29]

Polyhexamethylene Guanidine Phosphate. Beginning in 2006, epidemics of a fatal lung injury were observed in Korea every spring.[59] It was subsequently demonstrated that this type of children's interstitial lung disease (chILD), characterized by rapid progression and high mortality, was associated with humidifier disinfectant use. These disinfectants contain oligo (2- (2-ethoxy)ethoxyethyl) guanidium chloride, polyhexamethylene guanidine (PHMG), 5-chloro-2-methylisothiazol-3(2H)-one/2-methylisothiazol-3-(2H)-one, and didecyldimethylammonium chloride. Polyhexamethylene guanidine (not the ingredient that is under review in this safety assessment) has some chemical similarity with Polyaminopropyl Biguanide. The 2 chemical structures are presented below. Polyhexamethylene guanidine contains guanidine as part of its chemical structure, whereas Polyaminopropyl Biguanide contains biguanide.

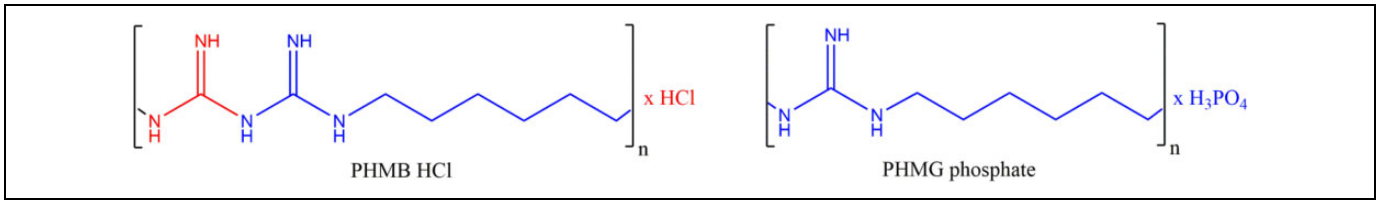

Figure 2. Polyaminopropyl Biguanide (PHMB HCl) versus PHMG phosphate.

The clinical characteristics of suspected cases between 2006 and 2011 were determined by a nationwide retrospective epidemiological study. The potential causal relationship with humidifier disinfectants was examined by a prospective surveillance study after humidifier disinfectant sales were suspended. One-hundred thirty-eight children (average age = 30.4 months) were diagnosed with chILD. The annual incidence increased in 2011 and then decreased to zero in 2012. At the time of hospital admission, the most frequent symptoms were cough and dyspnea. Disease progression resulted in spontaneous air leak and 80 children (58%) died. No new cases were found 2 years after the sale of humidifier disinfectants was suspended. The authors noted that the results of this study suggest that humidifier disinfectant inhalation causes an idiopathic type of chILD that is characterized by spontaneous air leak, rapid progression, lack of response to treatment, and high mortality.

A case–control study, with community-dwelling controls, was performed to validate the preceding study's findings and to confirm the exposure-response relationship between humidifier disinfectant and lung injury.[60] This study was based on reexamination of lung CAT scans and medical records at a hospital in Korea where many of the cases appeared. The purpose of the reexamination was to identify all cases of lung injury that fit certain criteria (ie, criteria for the type of lung injury that was associated with the use of humidifier disinfectants in the previous studies). Each case of lung injury was matched with 4 community-dwelling controls, according to age ($\pm$ 3 years), sex, residence, and history of childbirth since 2006 (for women). Using a questionnaire, environmental risk factors, which included the humidifier (type and use) and the humidifier disinfectant, were investigated in August of 2011. Exposure to the humidifier disinfectant was calculated for both cases and controls, and the corresponding risks of lung injury were compared. Sixteen patients who were among the 28 eligible cases agreed to participate. Sixty matched controls (selected from the community that the hospital serves) were considered eligible for participation in the study.

Study results indicated a statistically significant, exposure-response relationship between humidifier disinfectant exposure and lung injury. The cases were significantly more likely to have been exposed to humidifier disinfectants, compared to controls (odds ratio [OR]: 116.1; 95% confidence interval [CI]: 6.5-2063.7). The OR for an association between use of a humidifier disinfectant in which the active ingredient was specifically PHMG and lung injury was even greater (OR: 203.8; 95% CI: 11.1-3724.1) suggesting that the lung injuries observed in people who used humidifier disinfectants were attributable to the use of humidifier disinfectants containing PHMG. All cases used several liquid humidifier disinfectant formulations that contained the same proportion of PHMG phosphate. The concentration of PHMG phosphate in the humidifier mist was not stated. Further examination of associations

between exposure (number of bottles of disinfectant used per month × duration of exposure as number of months used × volume per bottle of disinfectant/days/ month) and lung injury indicated a clear relationship between the magnitude of daily exposure to disinfectants containing PHMG and the magnitude of the ORs. There was no association between lung injury and use of humidifier disinfectants in which the active ingredient was a combination of isothiazolinone derivatives (5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one [MCI/MI]) or a guanidinium derivative (oligo(2-(2-ethoxy)ethoxyethyl guanidinium chloride [PHG]).[60]

An analysis of patients and fatalities attributed to inhalation exposure to PHMG indicates that this chemical mainly causes lung diseases, such as pulmonary fibrosis.[61] Of the known main components of the humidifier disinfectants, PHMG has been identified as the chemical substance that caused the most deaths. In surveys conducted to identify victims of the humidifier disinfectant, 22% of the research participants answered that they had used the humidifier disinfectant, and 21% complained of side effects. According to another source, hundreds of individuals died from fatal lung injuries associated with use of humidifier disinfectants in Korea from 2002 through 2015.[62] Humidifier disinfectants containing PHMG were the most frequently used among confirmed humidifier-associated lung injury patients (n = 123, 55.7%). The development of humidifier-associated lung injury was found, clinically, to be associated with the use of several humidifier disinfectant products containing PHMG or other humidifier disinfectants.

In a refined risk assessment,[63] the time-weighted average PHMG concentration in the bedroom air was 0.06 mg/m^3 (calculated value, which is 250 times greater than the exposure concentration of Polyaminopropyl Biguanide at 0.00024 mg/m^3, which is derived from a MOS of 100) for this scenario, averaged over 8 hours. (The 28-day inhalation study on Polyaminopropyl Biguanide, summarized earlier in this safety assessment, was used as a comparison for the PHMG humidifier exposures.[18]) This concentration in air is 27 times greater than the 0.0022 mg/m^3 (the exposure concentration of Polyaminopropyl Biguanide that yields a MOS of 11) inhalation exposure concentration of Polyaminopropyl Biguanide estimated for the use of a pump hair spray containing the highest maximum reported concentration of use (0.053%) Polyaminopropyl Biguanide (see Table 9 in the safety assessment report). Further, the exposure duration of 8 hours for PHMG in the humidifier use scenario is 96 times greater than the conservative 5-minute exposure duration/event assumed for Polyaminopropyl Biguanide in the consumer spray scenarios evaluated in the safety assessment.

Summary

The safety of Polyaminopropyl Biguanide, which is used as a preservative in cosmetics, is reviewed in this assessment. Polyaminopropyl Biguanide is an INCI name; it refers to the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units (*chemical name*, polyhexamethylene biguanide hydrochloride [PHMB HCl]). It is not synonymous with the substance identified by the *chemical name* polyaminopropyl biguanide.

Polyaminopropyl Biguanide, in its neat form, represents a solid/powder of > 94.2% purity, and is usually marketed as an approximately 20% aqueous, preformulation solution. One method for manufacturing Polyaminopropyl Biguanide is via the polycondensation of sodium dicyanamide and hexamethylenediamine.

The following chemicals have been reported as possible impurities of Polyaminopropyl Biguanide: *N*-(6-aminohexyl)-*N*′-(6-(6-guanidinohexyl)guanidine, *N*-cyano-*N*′-(6-*N*-cyanoaminohexyl)guanidine, *N*-cyano-*N*′-(6-amnohexyl)guanidine), *N*-cyano-*N*′-6-(6-guanidinohexyl) guanidine hydrochloride, and 1,6-diguanidinohexane dihydrochloride.

According to 2019 VCRP data, Polyaminopropyl Biguanide is being used in 147 cosmetic formulations, which are mostly leave-on products. The results of a concentration of use survey provided in 2017 indicate that Polyaminopropyl Biguanide is being used at concentrations up to 0.2% in leave-on products (eye lotions), and use in baby lotions, oils, and creams (leave-on products) at concentrations up to 0.1% is also being reported. The highest maximum use concentration that is being reported for Polyaminopropyl Biguanide in rinse-off products is 0.1% in hair dyes and colors and in skin cleansing products. Polyaminopropyl Biguanide in reported to be used in aerosolized products according to VCRP data, but this type of use was not reported in the concentration of use survey.

In 2017, the SCCS issued a final opinion stating that the use of Polyaminopropyl Biguanide as a preservative in all cosmetic products at concentrations up to 0.1% is safe and that its use in sprayable formulations is not advised.

The safety of Polyaminopropyl Biguanide has been reviewed by the US EPA. The Agency concluded that this pesticide has very low aggregate risk of adverse health effects to the public or environment.

The results of a dermal penetration study on Polyaminopropyl Biguanide indicated that absorption through the skin equaled 1.56% (dermis contained 1.56% of applied dose) + 0.03% (receptor fluid contained 0.03% of applied dose). Based on SCCS Notes of Guidance, one standard deviation (2.5%) was added to the absorbed amount, yielding a calculated dermal absorption value of 4.09% (1.56% + 0.03% + 2.5% = 4.09%).

The principal route of excretion of radioactivity from orally administered (radiolabeled) Polyaminopropyl Biguanide was in the feces in the majority of the rat studies. However, in one rat study, most of the [^{14}C] Polyaminopropyl Biguanide administered in drinking water or in the diet was absorbed; and the majority was excreted in the urine, with lesser amounts excreted in the feces. The following components have been detected in the urine of rats fed Polyaminopropyl Biguanide in the diet: oligomers with 2 cyanoguanidino end groups, as well as the trace constituents, 3,3-dicyano-1,1-hexamethylenediguanidine and 1-(6-aminohexyl)-3-cyanoguanidine.

There was no incidence of mortality or systemic toxicity in rats that received a single dermal dose of 5000 mg/kg aqueous

Polyaminopropyl Biguanide; but, hemorrhage of dermal capillaries at the application site was observed. In an acute dermal toxicity study on 20% aqueous Polyaminopropyl Biguanide involving rabbits, an $LD_{50} > 400$ mg/kg was reported.

An LD_{50} of 1040 mg/kg was reported in a study in which rats were dosed orally with Polyaminopropyl Biguanide (concentration not stated) in distilled water. LD_{50} values of > 1000 mg/kg were reported for rats dosed orally with aqueous solutions of up to 25% Polyaminopropyl Biguanide. A median lethal dosage of 25.6 mg/kg was reported for rats dosed orally with a 0.4% Polyaminopropyl Biguanide solution.

LC_{50} s of > 0.36 mg/L and equal to 0.37 mg/L were reported in acute inhalation toxicity studies in which rats were exposed for 4 hours to Polyaminopropyl Biguanide (99.6%) solutions at concentrations of 360 mg/m^3 in air and up to 300 mg/m^3 in air, respectively. Dark/red lungs were observed at necropsy. A concentration-related depression of respiratory rate was reported in a study in which mice were exposed to Polyaminopropyl Biguanide (20% aqueous) at concentrations up to 208 mg/m^3.

No specific systemic effects were observed after 25% aqueous Polyaminopropyl Biguanide was applied to the skin of rats for 3 alternating 24-hour periods. There was also no evidence of systemic toxicity in rats that received 6, 24 hours dermal applications of 20% Polyaminopropyl Biguanide (diluted with water to 0.04% active ingredient). There were no mortalities or signs of systemic toxicity in rats that received dermal applications of 20.2% aqueous Polyaminopropyl Biguanide at dosages up to 200 mg/kg daily over a 30-day period (21 applications total; NOAEL = 200 mg/kg/d). In a 21-day dermal toxicity study involving rabbits, there was no evidence of toxic effects on the skin after 20% aqueous Polyaminopropyl Biguanide (12,000 ppm solution (1 mL) was applied daily.

Gastrointestinal inflammation was observed in rats dosed orally with 25% aqueous Polyaminopropyl Biguanide (in distilled water; initially at 1 g/kg and subsequently at 0.5 g/kg) for 21 days.[21] A LOAEL of 0.1 mg/mL for 20% aqueous Polyaminopropyl Biguanide (in drinking water) was reported in 28-day oral toxicity studies involving rats and mice.

Rats (groups of 10) that received Polyaminopropyl Biguanide (in drinking water, up to 150 mg/kg) for 4 weeks experienced dehydration, clinical signs of rough coat and hunched posture, and body weight loss (all classified as severe). Across the 3 dose groups, 10 rats had to be terminated due to severe weight loss, whereas, the remaining rats eventually adapted and began to gain weight. Absolute liver weights in all dose groups were similar to the control group. Mild centrilobular hypertrophy in the liver was observed in some of the rats (all dose groups). In the same study, Polyaminopropyl Biguanide administered (in the diet, 4000 mg/kg) to rats for 4 weeks caused a statistically significant decrease in body weight and absolute liver weight. In this dietary group, there was no evidence of centrilobular hypertrophy in the liver. Also, there was no evidence of necrosis or inflammatory lesions in the liver when Polyaminopropyl Biguanide was administered in drinking water or in the diet. In a 60-day gavage study on Polyaminopropyl Biguanide involving rats, mild toxicity in the liver or kidneys was observed (by microscopic examination) at 2 mg/kg/d (dose equivalent to 0.2 mg/L of 0.4% solution of test substance), 8 mg/kg/d (dose equivalent to 0.4 mg/L of 0.4% solution of test substance), and 32 mg/kg/d (highest dose, equivalent to 1.2 mg/L of 0.4% solution of test substance). None of the animals died.

In 21-day and 28-day inhalation toxicity studies on Polyaminopropyl Biguanide involving rats, NOAECs of 0.025 mg/m^3 and 0.0239 mg/m^3 were reported, respectively. In the 21-day study, the animals were exposed (nose-only, concentrations up to 26 mg/m^3) to the test substance 5 days per week, 6 h/day. Slightly-to-moderately severe pneumonitis was observed at histopathological examination in rats exposed to 0.25 mg/m^3. Moderate to severe pneumonitis was observed in rats exposed to 2.75 mg/m^3, and severe nasal irritation and dyspnea were observed at a concentration of 12.5 mg/m^3. Additionally, all rats of the 12.5 and 26 mg/m^3 groups died. In the 28-day study (nose-only, concentrations up to 2.5 mg/m^3, 6 h/day, 5 days per week), squamous metaplasia was observed in the larynx of males and females exposed to 0.25 mg/m^3 and 2.5 mg/m^3, and tracheal inflammation was observed in males and females exposed to 2.5 mg/m^3. Pneumonitis and bronchitis were observed in the lungs of males and females exposed to 2.5 mg/m^3.

There were no treatment-related macroscopic postmortem findings in mice in a 90-day drinking water study of 20% aqueous Polyaminopropyl Biguanide (concentrations up to 0.3 mg/mL in drinking water),[3] and a NOAEL of 1000 ppm was reported for this ingredient in a 90-day feeding study on 20.2% aqueous Polyaminopropyl Biguanide in which mice received concentrations up to 4000 ppm in the diet. The following results were reported in 90-day oral toxicity studies on Polyaminopropyl Biguanide involving rats: no mortalities, but iron pigment/deposits were observed in Kupffer cells (at 1250 ppm [in one study on 25% aqueous Polyaminopropyl Biguanide] and 5000 ppm [in another study on 25% aqueous Polyaminopropyl Biguanide] in diet; and an NOAEL of 1000 ppm [in a third study on 20.2% aqueous Polyaminopropyl Biguanide]). An NOAEL of 5500 ppm was reported for Beagle dogs fed Polyaminopropyl Biguanide at concentrations up to 11,000 ppm in the diet for 90 days.

In an 80-week chronic toxicity study involving mice (dermal applications 5 days/week), a mortality rate of 75% was reported for the highest dose group (10% Polyaminopropyl Biguanide; 30 mg dose). The exophthalmos observed throughout the study was more severe in this group, compared with the other groups, but the results of histological examination of the eyes and gross and microscopic examination of the thyroids were negative. An NOAEL of 0.6 mg/mouse/day (15 mg/kg/d) was reported.

Mice were fed 20% Polyaminopropyl Biguanide in the diet (concentrations up to 1000 ppm; feeding 1 week prior to mating and during mating [males and females] and continuation of feeding throughout pregnancy and lactation [females]). The offspring of these mice were also fed 20% Polyaminopropyl Biguanide at concentrations up to 1000 ppm in the diet for 97 weeks. For parents and their offspring, feeding with the test substance did not cause macroscopic changes in the spleen or

liver. In a 104-week oral toxicity study on 20.2% Polyaminopropyl Biguanide involving rats, an NOAEL of 600 ppm (36 mg/kg/d) was reported. The SCCS used this NOAEL to calculate a MOS. This calculation was performed, assuming that all cosmetics contain 0.1% Polyaminopropyl Biguanide and a dermal absorption value of 4.09%, and using the NOAEL of 600 ppm (36 mg/kg/d; corrected to 3.1 mg/kg/d, based on 8.5% oral absorption) and a SED of 0.012 mg/kg/d; MOS values of 258 (based on cosmetic exposure estimate) and 227 (based on cosmetic exposure estimate + noncosmetic exposure estimate) were determined. An NOEL of 200 ppm for histopathologic changes was reported in a 122-week oral toxicity study involving rats fed 20% Polyaminopropyl Biguanide at concentrations up to 2000 ppm in the diet. Increased adrenal weight was reported for males and females at concentrations of 1000 ppm and 2000 ppm in the diet.

In a 26-week feeding study involving dogs, dietary concentrations of 1500 ppm and 4500 ppm 20% Polyaminopropyl Biguanide produced concentration-related hepatotoxicity and nephrosis. An NOAEL of 1500 ppm for 20.2% Polyaminopropyl Biguanide was reported in a 1-year feeding study involving dogs; treatment-related histopathological findings in the liver and kidneys were reported in the high-dose group. In this study, groups of animals were fed test-substance concentrations of 300 ppm, 1500 ppm, and 4500 ppm for up to weeks 11/12. The 4500 ppm concentration was reduced to 3000 ppm for the remainder of the study because high dose males exhibited unexpected signs of toxicity, including marked reddening/peeling of scrotal skin, loss of appetite, body weight loss, and/or indications of liver impairment in the form of elevated plasma alanine transaminase and/or aspartate transaminase activities.

An NOAEL of 10 mg/kg/d for developmental toxicity was reported in an oral dosing (by gavage on gestation days 6 through 15) study on 20% aqueous Polyaminopropyl Biguanide involving mice. In oral reproductive and developmental toxicity studies on 20% aqueous Polyaminopropyl Biguanide in rats, NOAECs of 1000 ppm (after feeding in diet on gestation days 1 through 20) and 1300 ppm (after feeding in diet during a 9-day premating period and until the 3rd generation) have been reported. An NOAEL of 2000 ppm for reproductive and development effects was reported in a study in which rats were fed (through 2 successive generations) with 20.2% aqueous Polyaminopropyl Biguanide at dietary concentrations up to 2000 ppm. Polyaminopropyl Biguanide has been classified as embryotoxic at a dosage rate of 100 mg/kg/d (rats; protocol not stated).

An NOAEL of 40 mg/kg/d for developmental toxicity has been reported in an oral dosing (20.2% aqueous Polyaminopropyl Biguanide by gavage on gestation days 8 through 20) study involving rabbits. Polyaminopropyl Biguanide (0.04% in PEG) has been classified as embryotoxic in rabbits at an oral dosage rate of 32 mg/kg/d (animal strain and dosing protocol not stated). Polyaminopropyl Biguanide (concentration not stated) has been classified as teratogenic in rats at an intraperitoneal dosage rate of 10 mg/kg/d (dosing protocol not stated).[29] In an inhalation study on 20% aqueous Polyaminopropyl Biguanide, degeneration of seminiferous tubules in the testis of 1 male rat was observed after exposure to 0.25 mg/m^3 (6 h/day, 5 days/week for 3 weeks), but this was not observed in any other group, including the group exposed to the highest concentration (26 mg/m^3).

In Ames tests, ~20% Polyaminopropyl Biguanide was nongenotoxic at doses up to 5000 μg/plate, with and without metabolic activation. At the highest dose evaluated (333,300 μg/plate) in the Ames test, Polyaminopropyl Biguanide was weakly genotoxic in *Salmonella typhimurium* strain TA 1538 without metabolic activation. Polyaminopropyl Biguanide was nongenotoxic in a mouse lymphoma assays at concentrations up to 2000 μg/mL with and without metabolic activation, or in an in vitro micronucleus test (cultured human peripheral blood lymphocytes) at concentrations up to 50 μg/mL (without metabolic activation) and up to 250 μg/mL (with metabolic activation). In an in vivo micronucleus test, Polyaminopropyl Biguanide was nonclastogenic in polychromatic erythrocytes from mice that received single oral dosages up to 400 mg/kg. In an in vivo unscheduled DNA synthesis assay, there was no induction of unscheduled DNA synthesis in hepatocytes from rats that received single oral doses up to 1500 mg/kg.

Polyaminopropyl Biguanide (20%) was evaluated at concentrations up to 3000 μg/mL in the cell transformation assay (using baby hamster kidney fibroblasts); there was no difference in the number of transformed cell colonies between test and negative control cultures. In another assay, RAW 264.7 mouse macrophages (a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice) were cocultured with SVEC-10 mouse liver endothelial cells in various experimental conditions: preactivation of macrophages with Polyaminopropyl Biguanide or LPS and/or coculture in presence of Polyaminopropyl Biguanide. Polyaminopropyl Biguanide, tested at concentrations up to 1 ppm, had no direct effect on liver cell proliferation and did not potentiate cell proliferation induced by activated macrophages.

Polyaminopropyl Biguanide was classified as a hepatocarcinogen in mice at the highest dose tested in a study in which Polyaminopropyl Biguanide in ethanol was applied to the skin daily at doses up to 750 mg/kg/d (5 days/week) for 80 weeks. The NOAEL was 0.6 mg/mouse/day (15 mg/kg/d). A variety of inflammatory hepatic changes was observed in all groups, including the controls. However, at 750 mg/kg/d, severe hepatitis was observed in some of the animals. These hepatic changes appeared to have been mainly responsible for causing increased numbers of deaths in the high-dose group. A scientific advisory panel advising the SCCS indicated that the hepatitis observed in this study may be attributable to the *Helicobacter hepaticus* infections, which may also be responsible for the increased incidence of hepatocellular neoplasms in these animals.

Mice were fed 20.2% Polyaminopropyl Biguanide in the diet (up to 1000 ppm active ingredient; feeding 1 week prior to mating and during mating [males and females]). The offspring of these mice were also fed 20.2% Polyaminopropyl Biguanide at concentrations up to 1000 ppm active ingredient in the diet for 97 weeks. Except for vascular tumors, there were no treatment-related (nonneoplastic or neoplastic) increases in

histopathologic findings. Vascular tumors (hemangiosarcomas or hemangiomas) in the liver or other sites and a high mortality incidence (80%) were reported by week 97. A dose-related increase in the liver vascular tumor incidence was reported. After reviewing the results of this study, the SCCS concluded that the data are considered to be of low reliability due to the high mortality rate that was reported. When mice were fed Polyaminopropyl Biguanide at dietary concentrations up to 4000 ppm for up to 28 days, increased cell proliferation in a concentration-related manner was noted at 1200 ppm and 4000 ppm. In another study, a statistically significant increase in the incidence of hemangiosarcomas and hemangiomas was reported in male mice (C57B1/10J/CD-1 strain) that received Polyaminopropyl Biguanide at a dietary concentration of 4000 ppm daily for 2 years. A low incidence of hemangioma (2 of 64 males; 2 of 64 females) and hemangiosarcoma (1 of 64 females) was reported in a study in which rats were fed Polyaminopropyl Biguanide at a dietary concentration of 2000 ppm for 2 years.

In another 2-year study, Polyaminopropyl Biguanide was administered (in drinking water or in diet) to groups of rats. Hepatocellular tumors were induced at concentrations (in water) of 1000 mg/L and 1500 mg/L, but not at a concentration of 500 mg/L (in water). Administration of Polyaminopropyl Biguanide in diet did not cause increase in hepatocellular tumors. The hypothesized MOA for liver tumors induced by Polyaminopropyl Biguanide in drinking water involves increased hepatocyte proliferation and induction of hepatocellular foci and tumors. Polyaminopropyl Biguanide was classified as noncarcinogenic in rats fed dietary concentrations up to 2000 ppm for 122 weeks; mortality was 80%. In a feeding study in which rats were fed Polyaminopropyl Biguanide at concentrations up to 2000 ppm, 80% mortality was reported at 124 weeks. A low incidence of hemangiomas or hemangiosarcomas (mostly in lymph nodes) was observed in the groups of remaining animals (7 groups, with 8 to 21 rats/group; 1 animal with a hemangioma or hemangiosarcoma per group).

In a study involving A549 lung cells in vitro, it was noted that Polyaminopropyl Biguanide induces inflammatory responses via the NF-κB signaling pathway. Except for a slight increase in some cytokines and transcription factor at concentrations at which cell lysis occurs rapidly, Polyaminopropyl Biguanide did not exhibit clear and remarkable epigenetic properties at 20 to 100 μg/mL.

Polyaminopropyl Biguanide (PHMB) exhibited high antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, whereas, though chemically closely related, polyaminopropyl biguanide proved to be ineffective in bacterial eradication. When compared to Polyaminopropyl Biguanide (PHMB), polyaminopropyl biguanide displayed significantly lower cytotoxicity at concentrations ranging from 0.005% to 0.1% vol/vol; both chemicals were cytotoxic.

In a study involving mice, the highest dose of Polyaminopropyl Biguanide (10% concentration in ethanol, 30 mg dose) caused hyperkeratosis and, occasionally, ulceration extending into the dermis when applied repeatedly for 80 weeks.[21] Polyaminopropyl Biguanide (0.04%) was classified as a nonirritant when applied to the skin of rats for 24 hours. Repeated applications of 20.2% aqueous Polyaminopropyl Biguanide to rats for 21 days resulted in slight skin irritation (at 60 mg/kg/d) and moderate irritation (at 200 mg/kg/d). Severe skin irritation was observed in all rats that received a single 24-hour application of 25% aqueous Polyaminopropyl Biguanide at dosages of 2.5 mL/kg and 5 mL/kg. Focal ulceration and edema were observed in rats after 25% aqueous Polyaminopropyl Biguanide was applied repeatedly to the skin.

Repeated 23-hour applications of Polyaminopropyl Biguanide (12,000 ppm; 1 mL per application) to the skin of rabbits for 21 days were not irritating. Single applications (24 hours) of 20% aqueous Polyaminopropyl Biguanide to rabbits indicates that this compound is noncorrosive, but moderately irritating, to intact skin, and severely irritating to abraded skin. Polyaminopropyl Biguanide (20% aqueous) induced erythema at abraded, but not intact, skin sites in rabbits, and was classified as a noncorrosive material. Moderate erythema was observed after 20% aqueous Polyaminopropyl Biguanide was applied for 24 hours to the skin of rabbits. Mild skin irritation resulted after Polyaminopropyl Biguanide (96%, as powder) was applied to the skin of rabbits for up to 4 hours. Polyaminopropyl Biguanide (0.5 g, moistened with water; single 4-hour application) was also classified as a mild skin irritant in rabbits. Slight to moderate erythema was observed in guinea pigs that received repeated applications of 25% aqueous Polyaminopropyl Biguanide for 3 days.

Polyaminopropyl Biguanide (up to 1.5% active) was not classified as a primary skin irritant when applied for 24 hours to the skin of human subjects (17 males and 28 females).[18] A bacterial, nonocclusive dressing loaded with 1% wt/vol sericin and 0.3% wt/vol Polyaminopropyl Biguanide was classified as nonirritating after application to the skin of 105 subjects for 3 days.[34]

In a guinea pig maximization test on Polyaminopropyl Biguanide, moderate skin sensitization was observed in guinea pigs challenged with Polyaminopropyl Biguanide (20.2% active ingredient) and a 30% solution of the ingredient (6% active ingredient) in deionized water. The challenge phase was preceded by induction with 0.06% active ingredient (intradermal injection) and 20.2% active ingredient (occlusive application). Moderate to strong sensitization was observed in guinea pigs challenged with Polyaminopropyl Biguanide (20.2% active ingredient). The challenge phase was preceded by induction with 0.2% active ingredient (intradermal injection) and 20.2% active ingredient (topical application). In another guinea pig maximization test, sensitization was not observed in guinea pigs induced with 0.15% (intradermal injection) and 20% Polyaminopropyl Biguanide (topical application), followed by challenge with 10% or 20% Polyaminopropyl Biguanide.

In one Buehler test on Polyaminopropyl Biguanide, guinea pigs were induced with 2% active ingredient (topical application), challenged with 2% active ingredient, and rechallenged with 0.2%, 2%, and 4% active ingredient. The initial challenge with 2% active ingredient and rechallenge with 2% and 4%

active ingredient resulted in faint erythema; rechallenge with 0.2% active ingredient produced negative results. Polyaminopropyl Biguanide (2% active ingredient) was classified as a moderate sensitizer. In another Buehler test, it was determined that the threshold for eliciting sensitization in guinea pigs was ~ 1%. Induction concentrations ranged from 0.3% to 5% and challenge concentrations ranged from 0.075% to 15%. Results from a study evaluating the possible cross-reactivity of Polyaminopropyl Biguanide (challenge with 20%) with chlorhexidine (challenge with up to 4% chlorhexidine gluconate) in guinea pigs were negative.

In an HRIPT (191 subjects) 20% aqueous Polyaminopropyl Biguanide (2% active ingredient; effective concentration = 0.4%) did not cause primary skin irritation, but did result in sensitization. When a leave-on product containing 20% Polyaminopropyl Biguanide (tested at 0.5%, effective concentration 0.1%) was evaluated in an HRIPT involving 207 subjects, it was concluded that the product did not induce dermal sensitization. In another HRIPT (115 subjects; any ethnicity, provided that their degree of skin pigmentation did not significantly interfere with evaluations) on a neck cream containing 0.2% Polyaminopropyl Biguanide, the product did not cause clinically meaningful irritation or sensitization.

The skin sensitization potential of 20% Polyaminopropyl Biguanide (diluted with distilled water to 1% vol/vol prior to testing; 750 $\mu g/cm^2$) was evaluated using semi-occlusive patches in an HRIPT involving 108 subjects (Asian [~2%], Bi-racial [~3%], Black [~ 23%], Caucasian [~ 33%], and Hispanic [~39%]; Fitzpatrick skin types not stated). The authors concluded that Polyaminopropyl Biguanide did not induce dermal sensitization in the subjects tested, and a NESIL (determined based on data from this HRIPT) of 750 $\mu g/cm^2$ was used to calculate a QRA on various product types. The authors concluded that Polyaminopropyl Biguanide did not induce dermal sensitization in the subjects tested. Using the results from this study, a NESIL of 750 $\mu g/cm^2$ was chosen and used to perform QRAs on products of various use types at maximum reported use concentrations.

Very strong irritation potential, but no significant photoirritancy, was reported in a study in which male rats were tested (dermal application) with Polyaminopropyl Biguanide at concentrations of 2% and 5%. When tested at a concentration of 1%, in 26 subjects, Polyaminopropyl Biguanide was essentially nonirritating and did not induce sensitization, phototoxicity, or photoallergenicity.

Case reports with sensitization reactions to Polyaminopropyl Biguanide (reported as an ingredient of wet wipes, contact lens cleansing solutions, wound irrigation solutions, preoperative antiseptics, and antimicrobial foam dressings) have been reported. The prick test was used to diagnose immediate contact urticarial reactions in 44 patients with eczematous dermatitis. A positive reaction was observed in 1 patient.

Undiluted and 25% aqueous Polyaminopropyl Biguanide were severe ocular irritants when instilled into unrinsed rabbit eyes. Polyaminopropyl Biguanide (20% aqueous) induced slight inflammation, and Polyaminopropyl Biguanide (0.04% active ingredient) was nonirritating to the eyes of rabbits. In a study in which 20% aqueous Polyaminopropyl Biguanide was instilled into human eyes and the eyes of rabbits in a temperature-controlled chamber, normal corneal morphology was observed at histological examination.

Discussion

The safety of Polyaminopropyl Biguanide for use as a preservative in cosmetics is reviewed in this safety assessment. Polyaminopropyl Biguanide is an INCI name; it refers to the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units (PHMB HCl). This ingredient does not actually contain the chemical polyaminopropyl biguanide, which has a 3-carbon chain in each monomeric repeat unit. Rather, the INCI name, Polyaminopropyl Biguanide, applies exclusively to chemical PHMB, which has a 6 carbon chain in each monomeric repeat unit, and is always supplied as the hydrochloride salt. The chemical polyaminopropyl biguanide is not a cosmetic ingredient.

There was no evidence of systemic toxicity following dermal exposure to 0.4% Polyaminopropyl Biguanide, which is greater than the 0.2% maximum reported cosmetic use concentration of this ingredient. Furthermore, the Panel noted that the dermal penetration of Polyaminopropyl Biguanide is minimal, considering that most of the compound remains in the epidermis and its distribution systemically is not a concern.

Overall, the available in vivo and in vitro genotoxicity data on Polyaminopropyl Biguanide in bacterial and mammalian cells are negative. The Panel noted that in vitro genotoxicity assays are difficult to interpret for microbial toxins such as the cytotoxic preservative Polyaminopropyl Biguanide. However, after reviewing the available data, the Panel determined that genotoxicity is not a concern. A low incidence of hemangiomas and hemangiosarcomas was reported in a study in which rats were fed Polyaminopropyl Biguanide at a dietary concentration of 2000 ppm for 2 years. The Panel noted that the vascular tumors observed in rats and mice were likely attributable to sustained hepatotoxicity (ie, a nongenotoxic mechanism) from high exposures (ie, doses above the maximum tolerated dose) that the Panel considered not toxicologically relevant to cosmetic use. Furthermore, the carcinogenicity study results reviewed are equivocal.

Results were classified as positive for Polyaminopropyl Biguanide in the LLNA. However, interpreting the study results is hampered by the absence of a reported EC3. Additionally, the Panel noted that Polyaminopropyl Biguanide is a sensitizer at 2%, and that elicitation occurs at a much lower concentration (0.2%) in animal studies. Based on the results of these studies, the Panel expressed concerns about sensitization potential. In response, industry performed a QRA.

A NESIL of 1000 $\mu g/cm^2$ was chosen and used to form the QRA for contact dermatitis with Polyaminopropyl Biguanide in cosmetics. This NESIL supports the use of this ingredient at concentrations of $\leq$ 0.1%. Among the human data that were used to derive the NESIL was an HRIPT involving 26 subjects

tested with 1% Polyaminopropyl Biguanide at a dose of 1000 μg/cm^2, the highest nonsensitizing dose in relation to all of the HRIPT data that were considered. However, the Panel noted the small subject population in this HRIPT (a test population of ≥ 100 subjects is usually preferred). Furthermore, in an HRIPT on a neck cream containing 0.2% Polyaminopropyl Biguanide (dose = 100 μg/cm^2) that involved more than 100 subjects, faint and pink reactions were observed at various times during challenge or during induction in some subjects, but the skin types evaluated were not sufficiently diverse. Based on these observations, the Panel suggested that the NESIL of 1000 μg/cm^2 may not be correct and determined that an HRIPT (with at least 100 subjects with a range of Fitzpatrick skin types) on Polyaminopropyl Biguanide at doses of 500 and 1000 μg/cm^2 is needed. In response to this data need, an HRIPT on 1% aqueous Polyaminopropyl Biguanide (dose = 750 μg/cm^2) involving 108 subjects (Asian [∼2%], biracial [∼3%], Black [∼ 23%], Caucasian [∼ 33%], and Hispanic [∼39%]; Fitzpatrick skin types not stated) was provided by the Council. Polyaminopropyl Biguanide (1%) did not induce dermal sensitization in any subjects tested. Using the results from this study, a NESIL of 750 μg/cm^2 was chosen and used to perform QRAs on products of various use types at maximum reported use concentrations. However, other data included in this CIR safety assessment indicate the potential for sensitization to Polyaminopropyl Biguanide, specifically in an LLNA, HRIPT, and in guinea pig maximization tests. Acknowledging the positive sensitization data on Polyaminopropyl Biguanide, the Panel noted that the potential for induction of skin sensitization varies depending on a number of factors, including the area of product application and final formulation; thus, formulators should assess the potential for final formulations to induce sensitization using a QRA or other accepted methodologies.

In addition to concerns relating to sensitization potential, the Panel also expressed concern over the existence of case reports of contact urticaria attributable to the use of Polyaminopropyl Biguanide in wound dressings. However, it was determined that contact urticaria would not be an issue in relation to cosmetic product applications after considering that this reaction was observed under the conditions of burn dressings on severely damaged skin. The Panel also determined that skin irritation potential at cosmetic use concentrations is not a concern, considering that Polyaminopropyl Biguanide (up to 1.5% active) was not classified as a primary skin irritant when applied for 24 hours to the skin of human subjects.

The Panel noted clinical studies relating to child deaths in South Korea that were associated with inhalation exposure from humidifiers that had been disinfected with a humidifier disinfectant containing polyhexamethylene guanidine phosphate (often referred to as polyhexamethylene guanidine; PHMG). Polyhexamethylene guanidine is not the same chemical as the cosmetic ingredient Polyaminopropyl Biguanide. However, in an abundance of caution, the Panel requested MOS calculations for Polyaminopropyl Biguanide inhalation exposure from cosmetic products that are sprayed.

A Council survey of maximum reported use concentrations by product category indicates that Polyaminopropyl Biguanide is no longer used in pump or propellant hair sprays. However, products categorized as "Tonics, Dressings, and Other Hair Grooming" that contain Polyaminopropyl Biguanide at maximum use concentrations of up to 0.1% are reported in the survey, and it is possible that products included in this category are sprays. Furthermore, 2019 FDA VCRP data indicate that Polyaminopropyl Biguanide is used in the Other Fragrance Preparations product category (use concentration data unavailable). Given the potential for incidental inhalation exposure, CIR performed a risk assessment using the ConsExpo Web Spray Model (Consumer Exposure Model, Web version 1.0.1). The maximum concentrations of use (0.0004% in propellant hair sprays and 0.053% in pump hair sprays) included in this risk assessment to estimate the inhalation exposure concentrations of Polyaminopropyl Biguanide during the use of cosmetic spray products were based on results initially submitted to the Council in response to a use survey (submitted to CIR on April 11, 2017). The ConsExpo Web Spray Model and an NOAEC (from a 28-day inhalation study in which rats were exposed, nose only, to Polyaminopropyl Biguanide in an aerosolized water solution for 6 h/day, 5 days/week) were used in the MOS calculations for inhalation exposure. Margin of safety values for pump hair sprays (MOS = 11) and propellant hair sprays (MOS = 200) were calculated. Exposure concentrations that would yield an MOS of 100 for propellant and pump hair sprays were also calculated.

After reviewing this risk assessment, the Panel noted that the exposure scenario in the 28-day inhalation study is not representative of consumer pump and propellant hair spray product use, and determined that data more relevant to consumer use are needed. The Panel also noted that there are potential safety issues relating to chronic ingredient inhalation exposure, potentially experienced by hairdressers, but acknowledged that evaluation of occupational safety is not within the purview of the Panel.

The Panel determined that the following data are yet needed in order to evaluate the safety of Polyaminopropyl Biguanide in cosmetic products which may be incidentally inhaled:

- Consumer use data on pump and propellant hair sprays, for use in determining the extent of exposure to Polyaminopropyl Biguanide during product use. As part of this data insufficiency, use concentration data on this ingredient in aerosolized products and the particle size that is associated with the spray product are needed if Polyaminopropyl Biguanide is used in products that could be inhaled (ie, in products which may result in incidental inhalation).

Conclusion

The Expert Panel for Cosmetic Ingredient Safety concluded that Polyaminopropyl Biguanide is safe in cosmetics in the present practices of use and concentration described in the safety assessment, when formulated to be nonirritating and nonsensitizing, which may be based on a QRA or other

accepted methodologies. The Panel also concluded that the data are insufficient to determine the safety of Polyaminopropyl Biguanide in products that may be incidentally inhaled.

Author's Note

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

Author Contributions

Johnson, W. contributed to conception, design, acquisition, analysis, interpretation, and drafted the manuscript; Heldreth, B. contributed to conception, design, acquisition, analysis, interpretation, drafted the manuscript, and critically revised the manuscript; Boyer, I. contributed to analysis and interpretation and critically revised the manuscript; Zhu, J. contributed to conception, design, analysis, interpretation, drafted the manuscript, and critically revised the manuscript; Bergfeld, W. F., Belsito, D. V., Hill, R. A., Klaassen, C. D., Liebler, D. C., Marks, J. G., Shank, R. C., Slaga, T. J., and Snyder, P. W. contributed to conception and design, contributed to analysis and interpretation, and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Table 1. Chemical Properties of Polyaminopropyl Biguanide.

Property	Value	Reference
physical form (at 20 °C and 101.3 kilopascals [kPa]) and color	Pale yellow powder	3
Average molecular weight (Da)	3686-4216. Molecular weight distribution in commercially used mixture: 6% is $<$ 500, 14.1% is between 500 and 1000, and 75.8% is $>$ 1000	3
Water solubility (g/100 mL)	41 $\pm$ 1	3
Other solubility (g/100 mL)	ethanol: 0.5 $\pm$ 0.08 methanol: 41 $\pm$ 1	3
Relative density (at 20 $\pm$ 0.5 °C)	1.20 $\pm$ 0.0025	3
Melting point (°C)	78.9-136.3	3
Boiling point (°C)	decomposes at 205-210, before boiling	3
Vapor pressure (Pa at 20 °C)	1.32×10^{-7}	3
Log P_{ow} (at 25 °C $\pm$ 1 °C)	-2.3	3
Ultraviolet absorption (λ) (nm)	236	3

Table 2. Frequency (2019) and Concentration (2017) of Use According to Duration and Type of Exposure.

	# of Uses[5]	Max Conc of use (%)[6]
	Polyaminopropyl Biguanide	
Totals*	147	*0.00001-0.2*
Duration of use		
Leave-on	*100*	*0.00001-0.2*
Rinse-off	*47*	*0.00025-0.1*
Diluted for (bath) use	NR	
Exposure type		
Eye area	27	0.01-0.2
Incidental ingestion	1	NR
Incidental inhalation-spray	1; 26[a]; 30[b]	NR; 0.000023-0.1[a]
Incidental inhalation-powder	30[b]	NR
Dermal contact	94	0.00001-0.2
Deodorant (underarm)	NR	0.003
Hair—Noncoloring	12	0.000023-0.1
Hair-coloring	NR	0.1
Nail	4	NR
Mucous membrane	11	0.006
Baby products	1	0.1

Abbreviation: NR, not reported.

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

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

[b] Not specified these products are sprays or powders, but it is possible the use can be as a spray or powder, therefore the information is captured in both categories.

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

Table 3. Dermal Penetration Studies.

Ingredient	Animals/Protocol	Results
[^{14}C]-Polyaminopropyl Biguanide (20.2% aqueous; specific activity = 0.88 mCi/mL)	Various concentrations applied to human skin (epidermis from abdominal skin) in diffusion cell (dose volume = 1 mL; receptor fluid; sterile physiological saline). Receptor fluid samples collected daily for up to 15 days. Also, uptake experiment whereby 2 cm^2 rat skin disks (whole skin from flank and dorsum of male and female Wistar-derived, Alderley-Park rats) bathed in different concentrations; 5-day equilibration phase.	At concentrations of 0.4%, 1.4%, 5%, and 20%, absorption rates (ng/cm^2/h) through human epidermis were 8.13, 22.8, 350, and 1005, respectively. At concentrations of 0.4%, 20% (early phase—not defined), and 20% (late phase—not defined) [^{14}C]-Polyaminopropyl Biguanide absorption rates (ng/cm^2/h) through rat whole skin were 131, 3695, and 11,940, respectively.[18]
[^{14}C]- Polyaminopropyl Biguanide (5% solution)	Applied to skin biopsies of newborn, hairless rats and to human epidermal skin in diffusion chamber (receptor fluid not stated).	For rat skin biopsies, no skin absorption was detected up to day 5 of exposure. For human epidermal skin biopsies, low rate of penetration of ~0.09% was noted after 24 hours, and this penetration rate was from 0.11% up to 0.81% after adding dimethyl sulfoxide (DMSO) to dosing solution.[18]
[^{14}C]-Polyaminopropyl Biguanide (0.1% wt/wt in aqueous micellar solution); [^{14}C]-Polyaminopropyl Biguanide (0.1% wt/wt in oil-in-water emulsion)	0.1% in aqueous micellar solution and 0.1% in oil-in-water emulsion, respectively, applied (24-hour exposure study) to human split-thickness skin from 4 donors (dose = 200 μL/cm^2; ≈ 2 mg/cm^2) in diffusion cell (receptor fluid: phosphate-buffered saline with sodium azide [0.01% wt/vol]). Penetration was determined directly after exposure.	Total dislodgeable dose (skin wash + tissue swab + pipette tip + donor chamber wash): 48.43% (for test substance in aqueous micellar solution) and 52.35% (for test substance in oil-in-water emulsion) of radioactivity removed during skin washing. At 24 hours post-dosing, absorbed (fraction of applied dose that was measured in receptor fluid) dose was 0.03% (for test substance in aqueous micellar solution) and 0.04% (for test substance in oil-in-water emulsion). The epidermis + lower layers of stratum corneum contained 11.47% (for test substance in aqueous micellar solution) and 14.20% (for test substance in oil-in-water emulsion) of the applied dose. The dermis contained 1.56% (for test substance in aqueous micellar solution) and 1.02% (for test substance in oil-in-water emulsion) of the applied dose. Mass balance was complete: 90.93% (for test substance in aqueous micellar solution) and 98.96% (for test substance in oil-in-water emulsion) of the applied dose. Based on SCCS Notes of Guidance, one standard deviation (2.5%) was added to the absorbed amount, yielding a calculated dermal absorption value of 4.09% (1.56% + 0.03% + 2.5% = 4.09%).[3]
[^{14}C]-Polyaminopropyl Biguanide (0.3% wt/wt in aqueous micellar solution); [^{14}C]-Polyaminopropyl Biguanide (0.3% wt/wt in oil-in-water emulsion)	Polyaminopropyl Biguanide solutions applied to human split-thickness skin from 4 donors (dose volume = 200 μL/cm^2; application rate ≈ 2 mg/cm^2) in diffusion cell (receptor fluid: phosphate-buffered saline with sodium azide [0.01% wt/vol]). In Part 1, penetration of the 0.1% aqueous micellar solution and 0.1% in oil-in-water emulsion determined directly after 24-hour exposure period. In Part 2, 24-hour exposure to 0.3% aqueous micellar solution and to 0.3% in oil-in-water emulsion followed by additional 72-hour period to determine whether test compound absorbed into the skin during previous 24-hour period would move from skin into the receptor fluid after the washout. All samples analyzed by liquid scintillation counting.	In 24-hour study, 48.43% (from aqueous solution) and 52.35% (from oil/water emulsion) of [^{14}C]-Polyaminopropyl Biguanide-derived radioactivity removed during washing procedure (dislodgeable dose at 24 hours). At 24-hours post dose, absorbed (fraction of applied dose measured in receptor fluid) dose was 0.03% (0.58 ng equiv/cm^2, from aqueous solution) and 0.04% (0.72 ng equiv/cm^2, from oil/water emulsion) of the applied dose. Epidermis + lower layers of stratum corneum contained 11.47% (238 ng equiv/cm^{2n}, from aqueous solution) and 14.20% (291 ng equiv/cm^2, from oil/water emulsion) of applied dose. Dermis contained 1.56% (32.3 ng equiv/cm^2, from aqueous solution) and 1.02% (20.9 ng equiv/cm^2, from oil/water emulsion) of applied dose. In the 72-hour study, 53.33% (from aqueous solution) and 58.10% (from oil/water emulsion) of [^{14}C]-Polyaminopropyl Biguanide-derived radioactivity was removed during washing procedure. At 72 -hour post dose, absorbed dose was 0.02% (1.29 ng equiv/cm^2, from aqueous solution) and 0.03% (1.94 ng equiv/cm^2, from oil/water emulsion) of applied dose. Epidermis + lower layers of stratum corneum contained 14.54% (972 ng equiv/cm^2, from aqueous solution) and 14.45% (921 ng

(continued)

Table 3. (continued)

Ingredient	Animals/Protocol	Results
		equiv/cm^2, from oil/water emulsion) of applied dose. Dermis contained 1.23% (82.0 ng equiv/cm^2, from aqueous solution) and 1.46% (93.4 ng equiv/cm^2, from oil/water emulsion) of the applied dose. Absorption through skin = 1.56% (dermis contained 1.56% of applied dose) + 0.03% (absorbed dose = 0.03% of applied dose). Based on SCCS Notes of Guidance, one standard deviation (2.5%) added to absorbed amount, yielding calculated dermal absorption value of 4.09% (1.56% + 0.03% + 2.5% = 4.09%).[3]
[^{14}C]-Polyaminopropyl Biguanide (19.2% aqueous; specific activity = 38.9 mCi/g; tested at 0.3% wt/wt in representative cosmetic formulation)	Polyaminopropyl Biguanide formulation applied to human split-thickness skin from 5 donors in diffusion cell (receptor fluid: phosphate-buffered saline). Application up to 24 hours.	At 24 hours, the absorbed dose (mean: 0.17%) was the sum of the receptor fluid (0.171%) and the receptor wash (definition not provided, 0.01%). Dermal delivery (3.49%) was the sum of the absorbed dose and the portion in the epidermis (3.18%) and the dermis (0.14%).[18]
[^{14}C]-Polyaminopropyl Biguanide (20.2% aqueous; specific activity = 1.85 gigabecquerel [GBq]/732 mg)	Applied to human skin epidermal membranes in diffusion cell (receptor fluid: distilled water). Nominal concentrations up to ~200 g/L applied (not occluded) at 10 μL/cm^2. ~200 g/L also applied (occluded) at 200 μL/cm^2.	At ~200 g active ingredient/L (occluded), absorption rate 0.110 ± 0.044 μg/cm^2/h (n = 4) and absorption percentage 0.001% over 24 hours. At 197 g active ingredient/L (unoccluded), absorption rate 0.009 ± 0.003 μg/cm^2/h (n = 5) and absorption percentage 0.012% over 24 hours.[18]
20.2% aqueous Polyaminopropyl Biguanide (20.2% aqueous; specific activity = 1.4 MBq/mg)	Test substance warmed to 40 °C and nominal concentrations up to 200 g/L applied (at volume of 10 μL/cm^2, unoccluded and occluded) to human skin epidermal membranes in diffusion cell (receptor fluid: distilled water).	At a concentration of 200 g active ingredient/L (occluded for 0.5 hours then unoccluded for 23.5 h), absorption rate was < 0.002 ± < 0.001 μg/cm^2/h (n = 6) and absorption percentage was < the limit of quantitation over a 24-hour period. Other data for a dose of 200 g active ingredient/L (occluded) indicated an absorption rate of 0.118 ± 0.012 μg/cm^2/h (n = 5) and an absorption percentage of 0.007% over a 24-hour period.[18]

Abbreviation: SCCS, Scientific Committee on Consumer Safety.

Table 4. Toxicokinetics Studies—Oral Exposure.

Ingredient	Animals/Protocol	Results
Polyaminopropyl Biguanide (in drinking water or in diet)	Total of 6 groups in study. Four groups of 4 male Wistar Han rats treated with [^{14}C]-Polyaminopropyl Biguanide at doses of 500, 1000, 1500 mg/L (in drinking water) and 4000 mg/kg (in diet) for 7 days. Additionally, a recovery study included 2 groups of 4 rats treated with [^{14}C]-Polyaminopropyl Biguanide at doses of 1500 mg/L (in drinking water) and 4000 mg/kg (in diet) for 5 days. Dosing was followed by withdrawal of treatment for 5 days. At $\sim$ 24 hours prior to termination, 24-hour urine and feces collected in metabolism cages. At necropsy, blood collected from the abdominal aorta, and the liver, kidneys, stomach and testes were removed.	Most of the [^{14}C]-Polyaminopropyl Biguanide administered in the drinking water or in the diet was absorbed, and the majority was excreted in the urine, with small amounts excreted in the feces. For the 3 groups that received test substance in drinking water, [^{14}C]-Polyaminopropyl Biguanide levels in feces and urine conformed to a clear dose response. The data indicated that total DPM (disintegrations per minute) consumed considerably more in rats that received Polyaminopropyl Biguanide in the diet when compared to drinking water. Low levels of radioactivity present in whole blood, with even lower levels in the plasma, liver, kidneys, stomach, and testes. DPM significantly lower in urine and feces of recovery groups. Recovery groups also had reduced amounts of radioactivity in whole blood and low levels of radiolabel remained in tissues, particularly in the kidneys. Thus, most of the Polyaminopropyl Biguanide administered in drinking water or in the diet was absorbed, and the majority was excreted in the urine, with lesser amounts excreted in the feces.[20]
[^{14}C]-Polyaminopropyl Biguanide (20% aqueous in double deionized water; specific activity = 1.85 GBq/4 mmol)	Groups of Alpk: APfSD (Wistar-derived) rats (3 to 5/sex/group). Single oral dosage (20 mg/kg) administered by gavage. Labelled and unlabeled test substances fractionated into low, medium, and high molecular weight (MW) fractions by centrifugation and also administered orally.	In bioavailability experiment (3 groups of 4 males), following single oral doses of low, medium, and high MW fraction of Polyaminopropyl Biguanide, 94.9%, 101.4%, and 96% of radioactivity, respectively, was eliminated via feces and 5.2%, 0.2%, and 0.2% of the radioactivity, respectively, was excreted via urine. In biliary excretion experiment (3 rats), single oral dose of unfractionated test substance administered: Most of radioactivity excreted via feces over 48 hours (96.8% in males; 98.9% in females), $<$ 3% excreted in urine, and $<$ 0.2% excreted in bile. In excretion and tissue retention experiments (5 males, 5 females), single oral dose of low MW fraction: Males excreted 7.8% via urine and 94.1% via feces; females excreted 2.6% via urine and 93.5% via feces. In tissues, highest amounts of radioactivity found in livers (0.18% of dose in males; 0.19% of dose in females) and kidneys (0.03% of dose in males; 0.04% of dose in females). Lower concentrations found in all other tissues investigated. Residual carcasses contained 0.22 and 0.28% of administered dose. It was noted that up to 8.5% of applied radioactivity might be considered bioavailable (sum of urinary excretion and radioactivity in tissues and residual carcass at study termination).[3]
[^{14}C]-Polyaminopropyl Biguanide (20% aqueous in double deionized water; specific activity = 1.85 GBq/4 mmol)	Groups of Alpk: APfSD (Wistar-derived) rats (5/sex/group) fed diets containing either 200 ppm or 2000 ppm unlabeled ingredient for 14 days. Groups then fed single oral dose of diet incorporating [^{14}C]-labeled ingredient as a 9% suspension (4 mL/kg). High dose corresponded to 0.8 mg [^{14}C]-labeled ingredient / kg (2 MBq/kg), and low dose to 0.08 mg [^{14}C]-labeled ingredient/ kg (0.2 MBq/kg).	Principal route of excretion of radioactivity was feces. At 200 ppm, fecal excretion of radioactivity amounted to 105% and 109% of administered dose for male and female rats, respectively. At 2000 ppm, percentages of fecal excretion were 106% and 105% in male and female animals. Urinary excretion accounted for 2.1% and 2.2% of dose in males and females at the low dose and for 2.3% and 1.8% in males and females at the high dose. Conclusion: At

(continued)

Table 4. (continued)

Ingredient	Animals/Protocol	Results
		200 ppm, 4.7% and 3.9% of administered doses bioavailable in males and females, respectively. Bioavailability 3.0% and 2.6% in high dose males and females, respectively. The major parts of radioactivity were excreted during the first 24 hour and excretion was virtually complete within 72 hours.[18]
Radiolabeled Polyaminopropyl Biguanide	5 male Alderley Park rats. Oral dosage (dosing method not stated) rate 20 mg/kg/d over 10 days.	5.6% $\pm$ 0.35% excreted in urine, 93.1% $\pm$ 1.58% excreted via feces, and 0.2% was exhaled.[18,19]
Radiolabeled Polyaminopropyl Biguanide	Male Alderley Park rats fed diet containing 20 ppm (duration not stated).	Greatest amounts of radioactivity detected in adipose tissue, followed by kidneys and livers. No radioactivity detected in brain. Urinary polymer-related material consisted of small amounts of Polyaminopropyl Biguanide oligomers with 2 cyanoguanidino end groups, as well as the trace constituents 3,3-dicyano-1,1-hexamethylenediguanidine and compound that was considered to be 1 (6-aminohexyl)-3-cyanoguanidine.[18,19]
20% [^{14}C]-Polyaminopropyl Biguanide (4.6 μCi)	5 male rats (strain not stated). Feeding with dosages of 100 mg/kg in the diet (duration not stated).	93% of radioactivity excreted in feces within 5 days. Six percent of radioactivity found in urine, 0.6% found in bile, and 0.2% found in expired air. Findings suggested to the authors that test substance was poorly absorbed from gut and no evidence of enterohepatic recirculation.[21]
20% [^{14}C]-Polyaminopropyl Biguanide	Groups of 3 male rats (strain not stated) fed diet that contained 100 ppm test substance	Concentration in abdominal fat peaked at 1.2 ppm after 3 weeks and was maintained at this level for another 2 weeks on diet. After returning to normal diet, concentrations in the abdominal fat reduced to 0.3 ppm after 5 weeks. Concentration in the liver did not exceed 0.6 ppm after 5 weeks of feeding, and was reduced to undetectable levels within 3 weeks of return to normal diet. Comparable concentrations (maximum) in the kidney and heart were 0.8 ppm and 0.1 ppm. Radioactivity not detected in brain.[21]
[^{14}C]-Polyaminopropyl Biguanide	10 NMRI mice received single oral dose of 2.0 mL by gavage and were then frozen in acetone at up to 48 hours post-dosing. Whole body autoradiography subsequently performed (additional details not provided).	No absorption detected[18]

Table 5. Acute Toxicity Studies.

Ingredient	Animals	Protocol	Results
		Dermal Studies	
Polyaminopropyl Biguanide (96% pure, in distilled water)	10 Sprague-Dawley rats (5 males, 5 females).	OECD Guideline 402. Clipped skin of trunk treated with single dose of 5000 mg/kg. Application site covered with semi-occlusive dressing for 24 hours. 14-day observation period.	No mortalities or systemic toxicity. Hemorrhage of dermal capillaries noted at treatment sites of 8 animals 1 and 2 days after dosing. [2,18]
Polyaminopropyl Biguanide (20% aqueous)	2 groups of 20 (10 males, 10 females/group) specific pathogen free (SPF) albino rats.	Topical application of test substance at doses of 2.5 mL/kg and 5 mL/kg, respectively. Test substance applied to intact skin and spread over area of ~ 1 in^2 Site covered with patch for 24 hours. 7-day observation period. Necropsy not performed.	No mortalities.[21]
Polyaminopropyl Biguanide (20% aqueous)	4 New Zealand White rabbits (2 males, 2 females).	OECD Guideline 402. Test substance (2 mL) applied to shaved area ($\sim 150 \times 130$ mm) of dorsolumbar region and held in place with occlusive dressing for 24 hours. 14-day observation period.	Dermal $LD_{50} > 2$ mL/kg, ie, > 400 mg/kg (active ingredient).[18]
		Oral Studies	
Polyaminopropyl Biguanide (0.4% in deionized water)	Groups of 10 Sprague-Dawley rats	Single dose by gavage (stomach tube). Dosages ranged from 2 mg/kg to 40 mg/kg.	Administration of 25.6 mg/kg dose, ie, 1.6 mL of 0.4% Polyaminopropyl Biguanide solution (equivalent to 6.4×10^3 mg/L of 0.1% solution) resulted in 50% mortality. $LD_{50} = 25.6$ mg/kg. Following signs observed at LD_{50}: inactivity, ataxia, diarrhea, hyperreflexia, and convulsive twitching. No histopathological lesions in heart and kidney samples. 30% of animals tested had mild hydropic changes in zone 1 of liver samples.[22]
25% aqueous Polyaminopropyl Biguanide	6 rats (3 males, 3 females; strain not stated)	Single oral dose of 4000 mg/kg (equivalent to 1000 mg/kg Polyaminopropyl Biguanide) by stomach tube.) 7-day observation period.	1 female rat died. Necropsy findings included generalized congestion with gastric distention and hemorrhage, and lympholysis. $LD_{50} > 1000$ mg/kg Polyaminopropyl Biguanide.[21]
Polyaminopropyl Biguanide (96% pure, in distilled water)	6 female Sprague-Dawley rats	OECD Guideline 425. Dosed by gavage with 550 or 2000 mg/kg (dose volume = 20 mL/kg).	All 3 rats dosed with 2000 mg/kg died. No deaths at dose of 550 mg/kg. Signs of systemic toxicity in 1 animal dosed with 2000 mg/kg, but not at 550 mg/kg. Abnormalities noted at necropsy of rats that died were hemorrhagic or abnormally red lung, dark liver, dark kidneys, hemorrhage or sloughing of the gastric mucosa, sloughing of the nonglandular epithelium of the stomach, and hemorrhage of the small intestine. No abnormalities at necropsy of rats that survived 14-day observation period. $LD_{50} = 1049$ mg/kg.[18]
25% aqueous Polyaminopropyl Biguanide	3 female rats (strain not stated)	Single oral dose (2000 mg/kg), followed by 7-day observation period.	No deaths and all organs appeared normal at necropsy.[21]
20% aqueous Polyaminopropyl Biguanide	groups of Alderley Park rats (5 / sex/dose)	OECD Guideline 401. Doses up to 5000 mg/kg (dose volume = 10 mL/kg) administered by stomach tube. 14-day observation period. Necropsy not performed.	Signs of toxicity did not persist beyond day 7 or 8. LD_{50} of 2747 mg/kg (males) and 2504 mg/kg (females), corresponding to $\sim$ 549 and $\sim$ 501 mg/kg (active ingredient), respectively.[18]
25% aqueous Polyaminopropyl Biguanide	6 rats (3 males, 3 females; strain not specified)	Single oral dose of 40,000 mg/kg	1 male rat died. Severe generalized congestion with dilatation of the stomach and mucosal hemorrhage were observed at necropsy. Microscopic examination revealed gastric inflammation, ulceration, and thymic lympholysis, but no other specific lesions.[21]

(continued)

Table 5. (continued)

Ingredient	Animals	Protocol	Results
		Inhalation Studies	
Polyaminopropyl Biguanide (20% aqueous in spa water)	Groups of 5 mice of the Alpk: APfCD-1 strain	Mice exposed (duration not stated) to aerosol. Target concentrations of 5, 50, and 200 mg/m^3, corresponding to analyzed concentrations 11.7, 62.9, and 208 mg/m^3, respectively; median aerosol sizes (MMAD) 2.52, 3.08, and 4.31 μM.	Mean respiratory rate depression was 12% ± 4%, 20% ± 7%, and 40 ± 15% for target concentrations of 5, 50, and 200 mg/m^3, respectively, and RD_{50} (concentration causing 50% depression in respiratory rate) 264 mg/m^3 (no sensory irritation) calculated.[18] Mortality rate not reported. The SCCS noted that this RD_{50} is outside of investigated concentration range and is of questionable reliability. SCCS also stated that the results of this study indicate that test substance should be considered a respiratory irritant.[18]
Polyaminopropyl Biguanide (purity 99.6%) in aqueous solution	Wistar CRL:(WI) rats (groups of 10; 5/sex/test concentration).	OECD Guideline 403-compliant study. Exposure levels (nose-only): 0.1, 0.3, and 0.5 mg/L (100, 300, and 50 mg/m^3) for 4 hours. Mass medium aerodynamic diameters: 1.49-2.20 μM, with GSD in 1.84-2.29 μM range.	1 male died 3 hours after exposure. Respiratory distress in all females and most males. Red mottled lungs in dead male and 2 other males on day 15. LC_{50} estimated at > 0.36 mg/L (360 mg/m^3) for Polyaminopropyl Biguanide.[18]
20.6% wt/wt Polyaminopropyl Biguanide	Alpk: APfSC rats (10 rats; 5/sex	Exposed (nose-only) for 4 hours to single dose of 1.76 mg/L (1760 mg/m^3) of formulation (corresponds to 0.36 mg/L [360 mg/m^3]) of Polyaminopropyl Biguanide (mass medium aerodynamic diameters: 1.8-2.0 μM, with a geometric standard deviation [GSD] of 2 μM)	1 male died 3 hours after exposure. Respiratory distress in all females and most males. Red mottled lungs in dead male and 2 other males on day 15. LC_{50} estimated at > 0.36 mg/L (360 mg/m^3) for Polyaminopropyl Biguanide, as tested[18]

Abbreviations: MMAD, mass median aerodynamic diameter; SCCS, Scientific Committee on Consumer Safety.

Table 6. Short-Term Toxicity Studies.

Ingredient	Animals	Protocol	Results
		Dermal Studies	
20% Polyaminopropyl Biguanide (diluted with water to 0.04% active ingredient)	5 female rats of Alderley Park strain.	0.04% applied (0.1 mL) to back on alternate days for total of 6 applications. No covering or test site covered with polyethylene secured with an adhesive plaster for 24 hours.	No evidence of systemic toxicity (with or without covering).[21]
25% aqueous Polyaminopropyl Biguanide	3 female rats (strain not specified).	Test substance applied (amount per cm^2 not specified) to intact skin of the back, under occlusive dressing, for 3 alternating 24-hour periods; ie, each application period followed by 24-hour nontreatment period.	No specific systemic effects were observed.[21]
20.2% aqueous Polyaminopropyl Biguanide	Groups of 10 (5 males, 5 females per group) rats of the Alpk: APfSD (Wistar-derived) strain	Three groups received applications (occlusive, on back) of 0 mg/kg, 20 mg/kg, 60 mg/kg, and 200 mg/kg, respectively, 6 hours per day for 30 days (21 applications total). Fourth group served as the control.	No mortalities and no overt clinical signs of toxicity up to the highest dose tested. No substance-related effects on body weight, food consumption, organ weights, hematology, or clinical chemistry. Gross pathology and histopathology revealed no evidence of systemic toxicity. NOAEL for systemic toxicity = 200 mg/kg/d.[18]
20% aqueous Polyaminopropyl Biguanide	6 female albino rabbits	12,000 ppm solution (1 mL) applied (unoccluded) to the back for 23 hours. Reapplied, beginning at 1 hour later, for total of 21 daily applications.	No evidence of toxic effects on the skin.[18]
		Oral Studies	
20% aqueous Polyaminopropyl Biguanide	Groups of 20 (10 males, 10 females per group) mice of the C57Bl/10JfAP/alpk strain	Four groups received concentrations of 0.1, 0.3, 0.6, and 1.2 mg/mL, respectively, in drinking water for 28 days.	One male in 0.3 mg/mL group found dead on day 13. Dose-related initial loss of body weight, reduction in food and water consumption, and continued reduction in body weight and water consumption (considered a palatability effect). Treatment-related decrease in liver weight for males given 0.6 and 1.2 mg/mL, probably associated with poor nutritional status. Because effects on body weight and water consumption at all dose levels, NOAEL could not be derived. LOAEL = 0.1 mg/mL.[18]
25% aqueous Polyaminopropyl Biguanide	14 rats (7 males, 7 females; strain not specified)	Administered orally for 21 days, initially at 1 g/kg and subsequently at 0.5 g/kg doses.	4 males and 2 females survived 21 days of dosing; toxic signs not reported. Necropsy findings: gastrointestinal irritation, severe gastric hemorrhage, ulceration, peritonitis, thymic atrophy, and generalized congestion. At microscopic examination of major organs, nonspecific changes consistent with gastrointestinal inflammation.[21]
20% aqueous Polyaminopropyl Biguanide	Groups of 16 (8 males, 8 females per group) rats of the Alpk: APfSD strain.	Four groups received concentrations of 0.1, 0.5, 1, and 2 mg/mL, respectively, in drinking water for 28 days.	Dose-related loss in bodyweight/body weight gain and reduced water and/or food consumption occurring predominantly during the first days of treatment (considered a palatability effect). Increased liver weight at 1 mg/mL, decreased liver weight at 2 mg/mL, and dose-related increase in kidney weight at all dose levels. NOAEL could not be derived. LOAEL = 0.1 mg/mL.[18]
Polyaminopropyl Biguanide (in drinking water and in diet)	Groups of 10 male Wistar Han [Crl: WI(Han)] rats	Three groups received Polyaminopropyl Biguanide (in drinking water) at doses of 500, 1000, and 1500 mg/L, respectively ($\sim$ 50, 100, and 150 mg/kg, respectively) for 4 weeks. A fourth group received Polyaminopropyl Biguanide (in powdered diet) at a dose of 4000 mg/kg for 4 weeks, and another group served as the control (no test substance exposure). Animals were killed at end of study and necropsied. Liver sections prepared for histopathology	Polyaminopropyl Biguanide (in drinking water) caused dehydration, clinical signs of rough coat and hunched posture, and body weight loss (all classified as severe). Across the 3 dose groups, 10 rats terminated due to severe weight loss. Remaining rats eventually adapted and began to gain weight. Absolute liver weights in all dose groups were similar to control group. Mild centrilobular hypertrophy in liver observed in some of the rats (all dose groups). Test substance administered in diet caused statistically

(continued)

Table 6. (continued)

Ingredient	Animals	Protocol	Results
			significant decrease in body weight and absolute liver weight. No evidence of centrilobular hypertrophy in liver. Also, no evidence of necrosis or inflammatory lesions in the liver after dosing with test substance (in drinking water or in diet).[20]
Polyaminopropyl Biguanide (in deionized water)	Groups of 6 Sprague-Dawley rats	60-day study. Dosage (by gavage) rates: Group 1: 2 mg/kg (equivalent to 0.2 mg/L of 0.4% solution of test substance); Group 2: 8 mg/kg/d (equivalent to 0.4 mg/L of 0.4% solution of test substance); and Group 3: 32 mg/kg/d (equivalent to 1.2 mg/L of 0.4% solution of test substance). Control group received deionized water	No mortalities. Signs of systemic toxicity noted 2 days after dosing in 1 animal dosed with 32 mg/kg, exhibiting lethargy, ataxia, decreased respiratory rate, labored respiration, ptosis, and tiptoe gait. 50% of rats dosed with 32 mg/kg had either mild hepatocyte cytolysis or feathery degeneration with or without increased lymphocyte infiltration. No visible gross pathological changes in heart, liver, and kidney samples. At 2 and 8 mg/kg, mild toxicity in 50% of liver samples and 50% of kidney samples examined microscopically. At 32 mg/kg, mild toxicity in 50% of liver samples examined microscopically (mild kidney toxicity in 1 rat). In control group, mild toxicity (at microscopic examination) in kidneys of 30% of animals.[22]
Inhalation Studies			
20% aqueous Polyaminopropyl Biguanide	Groups of 8 (4 males, 4 females per group) SPF albino rats of the Alderley Park strain.	Five groups exposed (nose-only) to 0.025mg/m^3, 0.25 mg/m^3, and 2.75 mg/m^3, 12.5 mg/m^3, and 26 mg/m^3, respectively, 6 hours per day (5 days per week; 3 weeks total). Exposure to atmospheres of respirable particles (MMAD < 7 μM)	At 0.25 mg/m^3: 1 rat died and signs of moderate nasal irritation and tachypnea in this group. Histopathological examination revealed: slightly-to-moderately severe pneumonitis; thymus glands of 3 male and 3 female rats with reduction in cortical thickness and depletion of lymphocytes. Patchy loss of cilia in tracheal epithelium of 3 rats. At 2.75 mg/m^3, signs of nasal irritation and dyspnea. Histopathological examination revealed a moderate to severe pneumonitis. Thymus glands with severe depletion of lymphocytes and loss of normal architecture. At 12.5 and 26 mg/m^3, all rats died. Severe nasal irritation and dyspnea at 12.5 mg/m^3. NOAEC $=$ 0.025 mg/m^3.[18]
19.2% aqueous Polyaminopropyl Biguanide	Groups of 10 (5 males, 5 females per group) rats of the Alpk: APfSD (Wistar-derived) strain	Three groups were exposed (nose-only) to concentrations of 0.025mg/m^3, 0.25 mg/m^3, and 2.5 mg/m^3, respectively, 6 hours per day (5 days per week; 28 days total). For satellite groups (0, 0.025, and 2.5 mg/m^3) the recovery period was 13 weeks. Target air concentrations of aqueous Polyaminopropyl Biguanide were 0.0239 mg/m^3 (MMAD range: 0.32-1.30 μM), 0.257 mg/m^3 (MMAD range: 0.48-5.06 μM), and 2.47 mg/m^3 (MMAD range: 0.67-1.67 μM)	No treatment-related deaths or clinical signs up to 2.5 mg/m^3. No toxicologically significant changes in hematology or blood clinical chemistry parameters. Lung weights slightly elevated for males and females exposed to 2.5 mg/m^3; thymus weights elevated in males only at 2.5 mg/m^3. No macroscopic treatment-related findings observed at postmortem examination. Squamous metaplasia seen in the larynx of males and females at 0.25 and 2.5 mg/m^3, and tracheal inflammation in males and females at 2.5 mg/m^3. Pneumonitis and bronchitis in the lung in males and females exposed to 2.5 mg/m^3, at end of exposure period and recovery period. NOAEC $=$ 0.0239 mg/m^3.[18]

Abbreviations: LOAEL, lowest observed adverse effect level; MMAD, mass median aerodynamic diameter; NOAEC, no observed adverse effect concentrations; NOAEL, no observed adverse effect level; SPF, specific pathogen free.

Table 7. Subchronic Toxicity Studies.

Ingredient	Animals	Protocol	Results
		Oral Studies	
20% aqueous Polyaminopropyl Biguanide	Mice of the C57BL/10JfAP/Alpk strain. 2 groups of 10 males and 10 females (1 test and 1 control)	90-day drinking water study. Test group dosed with 0.1 mg/mL during 1st week, 0.3 mg/mL during 2nd week, and 0.3 mg/mL from 3rd week until study termination.	Reduction in body weight gain and dose-related reduction in water consumption. No treatment-related macroscopic postmortem findings.[3]
20.2% aqueous Polyaminopropyl Biguanide	C57Bl/10JfCD-1 mice, 4 mice/sex/group	90-day dietary study. Concentrations: 0, 1000, 2000, 4000 ppm active ingredient (corresponding to about 0, 162, 328, 736 mg/kg/d active ingredient in males and 0, 224, 445, 963 mg/kg/d active ingredient in females) and 6000 ppm active ingredient (mg/kg/d dose not stated).	The exposure of mice to 6000 ppm was terminated due to high mortality. Marked effects on body weight gain and marked toxicity (specific effects not stated) at 4000 ppm. No treatment-related effects on liver and kidney weights and no gross or histopathological findings. NOAEL = 1000 ppm (corresponding to 162 mg/kg/d in male mice and 224 mg/kg/d in female mice) as NOAEL.[18]
25% aqueous Polyaminopropyl Biguanide	Alderley Park Wistar rats (number of animals not stated)	90-day dietary study. Concentrations of 0, 625, and 1250 ppm active ingredient.	No mortalities. At 1250 ppm, deposits of an iron-pigment in liver (in hepatocytes and Kupffer cells) observed in female rats. No toxicity findings after feeding with 625 ppm.[18]
25% Polyaminopropyl Biguanide	Young adult SPF Wistar rats (25 males, 25 females/group)	90-day dietary study. Concentrations of 0 ppm, 2500 ppm, and 5000 ppm in diet.	No deaths during the 90-day feeding period. No gross abnormalities or abnormalities in hematological parameters. No remarkable changes in organ/body weight ratios. Microscopic examination revealed unusual degree of iron pigment in liver cells and in Kupffer cells for females fed 5000 ppm in the diet. Iron pigment not observed in liver of rats fed 2500 ppm in the diet (detailed histopathological results not included). Not possible to establish NOAEL.[21]
20.2% aqueous Polyaminopropyl Biguanide	Wistar-derived rats (Alpk: APfSD strain), 4 rats/sex/group	90-day dietary study. Concentrations: 0, 1000, 2000, 4000, and 6000 ppm active ingredient (corresponding to approximately 0, 83.9, 171.5, 373.0, 556.1 mg/kg/d active ingredient in males and 92.3, 192.9, 409.8, 617.4 mg/kg/d active ingredient in females).	Beginning at 2000 ppm, increased hemoglobin and hematocrit in males. Kidney was target organ. Renal functional change in form of decreased urine volume and increased specific gravity at 2000, 4000, or 6000 ppm animals (more marked in males). Treatment-related increase in kidney weight apparent for males at 4000 ppm or 6000 ppm (toxicological significance not determined). NOAEL = 1000 ppm (corresponding to 83.9 mg/kg bw/day in male rats and 92.3 mg/kg/d in female rats).[18]
25% aqueous Polyaminopropyl Biguanide	Three groups of Beagle dogs (inbred strain from Alderley Park, Cheshire; 4 males, 4 females per group)	90-day dietary study. Concentrations in diet of 0 ppm, 5500 ppm (1375 ppm active ingredient as dietary admixture), and 11,000 ppm (2750 ppm active ingredient as dietary admixture).	No mortalities or signs of systemic toxicity or other adverse effects in treated or control animals. Results for hematological parameters and clinical blood chemistries unremarkable. Liver function test (for bromsulphthalein [BSP] retention) results indicated no test substance-related effect. No significant treatment-related variations in organ/body weight ratios or test substance-related gross pathology. Microscopic examination revealed slight hemosiderin deposits in 2 of 4 males fed 11000 ppm. NOAEL = 5500 ppm.[18,21]

Abbreviations: NOAEL, no observed adverse effect level; SPF, specific pathogen free.

Table 8. Chronic Toxicity Studies.

Ingredient	Animals	Protocol	Results
		Dermal Study	
Polyaminopropyl Biguanide	Four groups of SPF Alderley Park mice (50 males, 50 females/group)	Test substance (0.3 mL) administered daily at following doses 5 days per week for 80 weeks: 0 (in ethanol), 0.6 mg (0.2% test substance in ethanol), 6.0 mg (20% test substance), and 30 mg (10% test substance in ethanol).	High mortality rate (75% in males and females) in 30 mg/d group at the end of the study, compared to ~ 30% in other groups. Exophthalmos observed throughout study; more severe in 30-mg group. Keratitis in many of affected animals. At week 80, exophthalmos incidence of 10% (6% for males and 13% for females). Clinical and histological examination of eyes and orbital contents revealed no evidence of pathological abnormalities. Gross and microscopic examinations of the thyroids normal in large majority of cases. Tissues from other organs were also examined microscopically. The SCCS noted that the highest dose administered in this study exceeded the maximum tolerated dose, and that the NOAEL was 0.6 mg/mouse/day (15 mg/kg/d).[3,21]
		Oral Studies	
20% Polyaminopropyl Biguanide	Groups of 30 male and 60 female SPF mice of the Alderley Park strain	Lifetime feeding study. 4 groups fed dietary concentrations of 0 ppm, 100 ppm, 200 ppm, and 1000 ppm, respectively, for 1 week prior to pairing and during mating. Feeding of females continued throughout pregnancy and lactation. All offspring were weaned at 3 weeks of age, and at 5 weeks of age, 50 males and 50 females were selected from each group. Offspring fed same diets as parents throughout study. Study terminated at 97 weeks after selection of the offspring, ie, when the overall mortality had reached 80%.	After 18 months, mortalities in all groups comparable, though higher in males than in females. Increased liver weight in males and females fed 1000 ppm. For males fed 1000 ppm, mean spleen weight significantly higher when compared to controls; based on macroscopic examination of tissues, finding not test substance-related. Other nonneoplastic findings (specific findings not stated) also not test substance-related.[21]
20.2% aqueous Polyaminopropyl Biguanide	Groups of 128 rats of the Alpk: APfSD (Wistar-derived) strain (64 males, 64 females per group)	Test substance administered in diet daily (for 104 weeks) at concentrations of 0 ppm, 200 ppm, 600 ppm, and 2000 ppm (corresponding to 0, ~12.1, ~36.3, and ~126.1 mg/kg/d [males] and 0, ~14.9, ~45.3, and ~162.3 mg/kg/d [females]).	No treatment-related clinical signs, ophthalmoscopic findings, or effects on any hematological or urinalysis parameters throughout study. Slightly raised plasma alkaline phosphatase activity, predominantly in females receiving 2000 ppm, and a slightly increased incidence of hepatocyte fat and spongiosis hepatitis in males (at 2000 ppm). NOAEL = 600 ppm (36 mg/kg/d).[18]
20% Polyaminopropyl Biguanide	Four groups of SPF rats of the Alderley Park strain (60 males, 60 females per group)	122-week study. Dietary concentrations of 0 ppm, 200 ppm, 1000 ppm, and 2000 ppm, respectively. Study terminated at 124 weeks, ie, when 80% mortality occurred in control group and in experiment overall.	Cumulative mortality comparable between control and treatment groups. Slight anemia at 104 weeks in female rats of 2000 ppm group. Other hematological parameters comparable among groups. At 52 weeks, females fed 2000 ppm had increased kidney weight. Increased adrenal weight reported for males and females of 1000 ppm and 2000 ppm groups. No treatment-related findings at necropsy. At 52 weeks, 104 weeks, and study termination, microscopic examination revealed increase in incidence of histiocyte conglomerates in mesenteric lymph nodes of female rats fed 1000 ppm and 2000 ppm. The NOEL (for histopathologic changes) = 200 ppm.[21]

(continued)

Table 8. (continued)

Ingredient	Animals	Protocol	Results
20% Polyaminopropyl Biguanide	Four groups of adult Beagle dogs (4 males, 4 females per group)	26-week study. Dietary concentrations of 0, 500, 1500, and 4500 ppm, respectively.	Treatment-related histopathological changes reported for sections of the liver and kidneys from dogs fed 4500 ppm: bile stasis, focal hepatocellular degeneration and necrosis, and focal proximal tubular nephrosis. Thus, feeding with dietary concentrations of 1500 ppm and 4500 ppm produced concentration-related hepatotoxicity and nephrosis.[21]
20.2% aqueous Polyaminopropyl Biguanide	Groups of 8 Beagle dogs (4 males, 4 females per group)	Test substance administered daily (for 1 year) at dietary concentrations of 0 ppm, 300 ppm, 1500 ppm, and 4500 ppm (corresponding to 0, ~11, ~54, and ~169 or ~108 mg/kg/d) up to weeks 11 or 12, and the concentration was reduced to 3000 ppm thereafter.	Males dosed with 4500 ppm had marked reddening/peeling of scrotal skin, loss of appetite, body weight loss and/or indications of liver impairment in the form of elevated plasma alanine transaminase and/or aspartate transaminase activities. Low testes weight apparent in male survivor in 3000 ppm group. Treatment-related histopathological findings in skin (dermatitis of scrotum, chin and limbs) as well as in the liver, kidney (males only), and testes of the high-dose group. No treatment-related histopathological changes in dogs of 300 or 1500 ppm group. NOAEL = 1500 ppm.[18]
Polyaminopropyl Biguanide	Species not specified	Chronic toxicity study (protocol not described).	NOEL = 200 mg/kg/d.[29]
Polyaminopropyl Biguanide	Species not specified	2-year chronic toxicity study (protocol not detailed). Dosage weight: 100 mg/kg/d	No adverse effects.[29]

Abbreviations: NOAEL, no observed adverse effect level; NOEL, no observed effect level; SCCS, Scientific Committee on Consumer Safety; SPF, specific pathogen free.

Table 9. Exposure Concentrations and Margins of Safety (MOSs) for Hair Spray Products Calculated Using the Cons Expo Web Model (version 1.0.1).[27]

Exposure Scenario Assumptions (spraying toward person) and Spray Parameters not Specific to Spray Type[a]			
Direction of spraying:	Toward exposed person	Room ventilation rate[b]:	0.2/h
Exposure duration/event:	5 min	Cloud Volume:	0.0625 m^3
Room volume:	10 m^3	Density nonvolatile:	1.5 g/mL
Room height:	2.5 m	Inhalation cutoff diameter:	15 μm

Spray Parameters and estimates of Exposure Concentrations and MOSs Specific for Spray type							
Cosmetic spray type	Spray duration (s)	Weight fraction of Polyaminopropyl Biguanide (%)	Mass generation rate (g/s)[e]	Airborne fraction (g/g)[e]	Initial median aerosol droplet diameter (μm) (Coefficient of Variation)[e]	Mean event Polyaminopropyl Biguanide exposure concentration (mg/m^3)[g]	MOS (NOAEC[g]/Mean event exposure concentration)[h]
Propellant hair spray	14.4[a]	0.0004[d]	0.4	0.2	46.5 (2.1)	0.00012	200
	14.4[a]	0.00084	0.4	0.2	46.5 (2.1)	0.00024	100
Pump spray	14.4[c]	0.053[d]	0.1[f]	0.02[f]	2.7 (0.73)[f]	0.0022	11
	14.4[c]	0.0058	0.1[f]	0.02[f]	2.7 (0.73)[f]	0.00024	100
Propellant deodorant spray	10.2[a]	0.000055	0.45	0.9	8.3 (0.84)	0.00024	100

[a]Default assumptions and values published by RIVM (Rijksinstituut voor Volksgezondheid en Milieu—Dutch National Institute for Health and Environment.[8,25]
[b]Default room ventilation rate specified in REACH guidance (Chapter R.15 Consumer exposure estimation, ECHA 2012), as noted in RIVM report.[25]
[c]Spray duration for pump hair sprays assumed to be the same as the default for propellant hair sprays.
[d]Concentrations of use reported in PCPC Industry survey dated April 11, 2017.[6]
[e]Mass generation rate, airborne fraction, and initial aerosol droplet diameters default assumptions published by RIVM.[26]
[f]Spray parameter default values developed for pump toilet water sprays assumed adequate for calculating conservative estimates of exposures from pump hair sprays.
[g]Exposure concentration averaged over the exposure duration.
[h]No observed adverse effect concentration (NOAEC) = 0.024 mg/m^3 from study; rats exposed 6 h/day, 5 day/week for 28 days to aqueous aerosols (mass median aerodynamic diameter [MMAD] = 0.32-1.30 μm.[3]

Table 10. Developmental and Reproductive Toxicity Studies.

Ingredient	Animals	Protocol	Results
		Oral	
20% aqueous Polyaminopropyl Biguanide (in 0.5% aqueous polyoxyethylene [20] sorbitan monooleate)	Groups of 47 to 49 SPF mice of the Alderley Park strain mated (matings yielded groups of 21 pregnant mice). Group of 25 mice served as the control. Because of the poor fertility rate, the mating of more than 40 mice per group occurred in order to yield at least 21 pregnant mice per group.	Four groups received (by gavage) 10, 20, or 40 mg/kg/d (expressed as active ingredient) on gestation days 6 through 15 (mating day considered gestation day 0). Total volume administered = 0.1 mL per 10 g of body weight.	No mortalities or test substance-related adverse clinical signs. Gestational parameters such as implantation sites, pre- and postimplantation loss, litter size and weight, resorptions not influenced by test substance at any dose. 21 fetuses with external abnormalities that were not test substance-related. Indications of slight retardation of ossification from examination of forelimb and hindlimb digits and numbers of caudal vertebrae at 20 and 40 mg/kg/d. Maternal NOAEL = 40 mg/kg/d. Developmental NOAEL = 10 mg/kg/d.[18,28]
Polyaminopropyl Biguanide	Rats (number and strain not specified)	Rats dosed orally with 100 mg/kg/d.	Embryotoxic.[29]
20% aqueous Polyaminopropyl Biguanide	Groups of 30 Sprague-Dawley rats (10 males, 20 females per group).	Four groups received dietary concentrations of 0, 200, 650, and 1300 ppm (dietary levels adjusted for 20% active ingredient) during the 9-week premating period and until the 3 rd generation.	Evaluations of the various reproductive indices, sex ratios, and body weight data of fetuses taken by Caesarean section and the offspring maintained through weaning revealed no meaningful differences between the control and treated groups. Necropsy of weanlings did not reveal any compound-related gross pathology. No findings indicative of embryotoxicity or teratogenicity. NOAEC = 1300 ppm.[18,21]
20% aqueous Polyaminopropyl Biguanide	Groups of 20 rats of the Alderley Park strain	Four groups received dietary concentrations of 0, 200, 1000, and 2000 ppm (expressed as active ingredient; corresponding to approximately 0, 13, 54, and 112 mg/kg/d) on gestation days 1 through 20 (mating day considered gestation day 0).	No mortalities and no adverse clinical effects in any group. No dose-related effects observed on fetal or litter weights. Increase in extra ribs at 2000 ppm considered consequence of maternal toxicity. No further test substance-related effect on fetal morphology, including ossification of the skeleton, in any of the test groups. Maternal NOAEC = 200 ppm. Developmental NOAEC = 1000 ppm.[18]
20.2% aqueous Polyaminopropyl Biguanide	Groups of 52 rats (26 males, 26 females) of the Alpk: APfSD (Wistar-derived) strain.	Four groups received dietary concentrations of 0, 200, 600, and 2000 ppm corresponding to 0, ~23-24, ~70-71, and ~239-249 mg/kg/d in (males), and to 0, ~25-26, ~77-79, ~258-270 mg/kg/d (females) through 2 successive generations (including a 10-week premating period).	No evidence of an effect on reproductive parameters or on offspring growth and development at concentrations up to 2000 ppm. Systemic, parental NOAEL = 600 ppm. NOAEL for reproductive and offspring effects = 2000 ppm.[18]

(continued)

Table 10. (continued)

Ingredient	Animals	Protocol	Results
20.2% aqueous Polyaminopropyl Biguanide	Groups of 20 female New Zealand White rabbits	Four groups received oral dosages (by gavage) of 0, 10, 20, and 40 mg/kg/d on gestation days 8 through 20.	No effect on the number of fetuses, growth or survival in utero, except a slight increase in preimplantation loss observed at 40 mg/kg/d (21.8 $\pm$ 25.6 vs 13.1 $\pm$ 15.2 in controls) and a significant increase in postimplantation loss at 20 mg/kg/d (11.4 $\pm$ 19.7% vs 6.1 $\pm$ 8.4% in controls) attributed to an increase in early intrauterine deaths. No evidence of teratogenicity. Percentage of fetuses with unossified 5th sternebrae or with fused 4th and 5th sternebrae increased at 40/mg/kg/d, but results not considered test substance-related. Maternal NOAEL = 20 mg/kg/d. Developmental NOAEL = 40 mg/kg/d.[18]
Polyaminopropyl Biguanide (0.04% in polyethylene glycol)	Rabbits (number and strain not stated)	Oral dosing (test protocol not included)	Embryotoxic at 32 mg/kg/d.[29]
		Parenteral	
Polyaminopropyl Biguanide	Rats (number and strain not specified)	Rats dosed intraperitoneally with 10 mg/kg/d.	Teratogenic.[29]
		Inhalation	
20% aqueous Polyaminopropyl Biguanide	Groups of 8 (4 males, 4 females per group) SPF albino rats of the Alderley Park strain	In short-term toxicity study, 5 groups exposed (nose-only) to concentrations of 0.025, 0.25, 2.75, 12.5, and 26 mg/m^3, respectively, 6 hours per day (5 days per week; 3 weeks total).	At 0.25 mg/m^3, degeneration of a few seminiferous tubules in testis of 1 male rat.[18]

Abbreviations: NOAEC, no observed adverse effect concentrations; NOAEL, no observed adverse effect level; SPF, specific pathogen free.

Table 11. Genotoxicity Studies.

Ingredient	Strain/cell type	Assay	Dose/Concentration	Results
			In Vitro	
20% aqueous Polyaminopropyl Biguanide	*Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538	Ames test, with and without metabolic activation	333.3 mg (333,300 μg) per plate	Toxic at 333.3 mg per plate, particularly in strains TA98, TA100, and TA1535. Weakly genotoxic in strain TA1538 without metabolic activation.[18]
20% aqueous Polyaminopropyl Biguanide	*S typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538	Ames test, with and without metabolic activation	Doses up to 500 μg/plate	Nongenotoxic.[18]
19.6% aqueous Polyaminopropyl Biguanide (in DMSO)	*S typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538.	Ames test, with and without metabolic activation	Doses up to 5000 μg/plate	Nongenotoxic, with or without metabolic activation in all but one strain. In strain TA98, negative results without metabolic activation, but slight responses (2.1 × background) observed with metabolic activation. Nongenotoxic.[18]
20% aqueous Polyaminopropyl Biguanide	L5178Y TK+/- mouse lymphoma cells	Mouse lymphoma assay, with and without metabolic activation	Concentrations up to 100 μg/mL	At 50 and 100 μg/mL, cytotoxicity higher than that of positive controls. Nongenotoxic.[18]
20% aqueous Polyaminopropyl Biguanide	P388 (*tk*+/-) mouse lymphoma cell line	Mouse lymphoma assay, with and without metabolic activation	Concentrations up to 2000 μg/mL	2000 μg/mL was cytolethal and clear cytotoxicity noted at 1000 μg/mL, with and without metabolic activation. Nongenotoxic.[18]
19.6% aqueous Polyaminopropyl Biguanide	Cultured human peripheral blood lymphocytes from 2 volunteers	Micronucleus test	Concentrations up to 50 μg/mL without metabolic activation and concentrations up to 250 μg/mL with metabolic activation.	No chromosomal aberrations. Nongenotoxic.[18]
			In Vivo	
19.6% aqueous Polyaminopropyl Biguanide	1000 polychromatic erythrocytes (from C57BL/6JfCD-1/Alpk mice) scored for presence of micronuclei	Micronucleus test	Groups of 10 mice. Test substance administered (single dose, by gavage) at 0, 250, and 400 mg/kg (dosage volume = 10 mL/kg).	Nonclastogenic.[18]
19.6% aqueous Polyaminopropyl Biguanide	Alpk: APfSD (Wistar-derived) rat hepatocyte cultures exposed to [^{3}H]-thymidine	Unscheduled DNA synthesis assay	Test substance administered (single dose, by gavage) to 2-3 males per dose at 0, 750, and 1500 mg/kg (dosage volume = 10 mL/kg) for 4 hours or 12 hours.	No induction of unscheduled DNA synthesis.[18]

Table 12. Carcinogenicity Studies.

Ingredient	Animals/Cells	Protocol	Results
		In Vitro Studies	
20% aqueous Polyaminopropyl Biguanide (in DMSO)	Baby hamster kidney fibroblasts (BHK21/C13)	Cell transformation assay, with metabolic activation. Test substances dose range of 0.25-2500 μg/mL and 25-3000 μg/mL in separate experiments.	Cytotoxicity at 250 μg/mL and greater. No difference in number of transformed cell colonies between test and negative control cultures. Test substance did not induce cell transformation.[18]
Polyaminopropyl Biguanide	RAW 264.7 mouse macrophages cocultured with SVEC4-10 mouse endothelial cells.	Experiment 1: Preactivation of macrophages with Polyaminopropyl Biguanide (0, 0.75, and 1 ppm) or lipopolysaccharide (LPS) and/or coculture in the presence of Polyaminopropyl Biguanide. Endothelial proliferation analyzed by incorporation of bromodeoxyuridine (BrdU). Experiment 2 summarized below.	Polyaminopropyl Biguanide had no direct effect on liver endothelial cell proliferation and did not potentiate cell proliferation induced by LPS-activated macrophages.[3]
Polyaminopropyl Biguanide	RAW 264.7 mouse macrophages	Reactive oxygen species (ROS) assay. Macrophages cultured with Polyaminopropyl Biguanide (0, 0.75, and 1 ppm). Production of ROS in macrophages detected by measurement of fluorescence intensity after addition of dihydrorhodamine and by evaluation of tumor necrosis factor (TNF) α and interleukin (IL)-6 in cell culture medium, as quantified by the enzyme-linked immunosorbent assay.	No activation of macrophages.[3]
		Dermal Studies	
Polyaminopropyl Biguanide (up to 20% aqueous)	Four groups of SPF mice (50 males, 50 females/group) of the Alderley Park strain (Alpk: APfCD-1strain)	Test substance (0.3 mL) was administered dermally (nonoccluded) at the following doses 5 days per week for 80 weeks: 0 (in ethanol), 0.6 mg (0.2% Polyaminopropyl Biguanide in ethanol), 6.0 mg (20% Polyaminopropyl Biguanide) and 30 mg (10% Polyaminopropyl Biguanide in ethanol). The 0, 0.6, 6, and 30 mg doses corresponded to 0, ~15, ~150, and ~750 mg/kg/d.	Incidence of clinically observed skin tumors: control (1 male), 6 mg of 20% concentration (2 males), and 30 mg/d of 10% concentration (1 male and 2 females). Liver + kidney tumors contributed more than 50% of total for the 30 mg/d group. Total number of kidney + liver tumors: control (5 males, 2 females), 0.6 mg/d group (4 males, 4 females), 6 mg/d group (5 males, 4 females), and 30 mg dose group (16 males, 7 females). Statistically significant increase in incidence of liver tumors (4 in controls and 10 in 30 mg/d group); statistically significant (Chi square, 1% level) only in case of liver tumors of endothelial origin (both benign and malignant; 2 in controls and 6 in 30 mg/d group). Many growths observed microscopically classified as moderate to severe hepatitis at histopathologic examination. Liver necrosis in all dose groups. Test substance classified as hepatocarcinogen in mice dosed with 30 mg/d. An NOAEL of 0.6 mg/mouse/day (15 mg/kg/d) was reported. A scientific advisory panel advising the SCCS indicated that the hepatitis observed in this study may be attributable to the *Helicobacter hepaticus* infections, which may also be responsible for the increased incidence of hepatocellular neoplasms in these animals.[21]
		Oral Studies	
20.2% aqueous Polyaminopropyl Biguanide	Groups of 30 male and 60 female SPF mice of the Alderley Park strain	Four groups fed diets containing 0, 500, 1000, or 5000 ppm (equivalent to 0, 100, 200, and 1000 ppm active ingredient, respectively) for 1 week prior to pairing and during mating. Offspring fed same diets as parents throughout experiment (97 weeks).	Study terminated when overall mortality reached 80% at 97 weeks (dosing time after selection of offspring). High mortality due to fighting of males. No treatment-related (nonneoplastic or neoplastic) increases in histopathologic findings. However, regarding vascular tumors of concern, there were some animals with hemangiomas or hemangiosarcomas in the liver or at other sites. Number of tumor-bearing animals: control (39 [18 males, 21 females]), 100 ppm (36 [16 males, 36 females]), 200 ppm (42 [17 males, 25 females]), and 1000 ppm (44 [23 males, 21 females]).

(continued)

Table 12. (continued)

Ingredient	Animals/Cells	Protocol	Results
			Liver neoplasms observed only in male mice and incidence was: control (2/39 =5.1%), 100 ppm (2/36 = 5.5%), 200 ppm (5/42 = 11.9%), and 1000 ppm (9/44 = 20.9%). Dose-related tumor incidence in liver.[21] According to the SCCS, these data were considered to be of low reliability due to high mortality.[18]
Polyaminopropyl Biguanide	Groups of 5 male C57Bl mice	Concentrations of 0, 100, 200, 400, 1200, and 4000 ppm in diet for 7, 14, or 28 days. Immunohistochemical detection of BrdU in mouse liver used to quantify cell proliferation in liver endothelial cells. Liver hepatotoxicity assessed by measuring alanine aminotransferase and aspartate aminotransferase in plasma of animals killed	Polyaminopropyl Biguanide increased cell proliferation in concentration-related manner at 1200 ppm and 4000 ppm. Cell proliferation also increased at 1200 ppm after feeding for 14 days. Plasma endotoxin, known activator of macrophages, increased at 1200 and 4000 ppm (after feeding for 28 days) and at 100 and 200 ppm (after feeding for 14 days).[3]
20.2% aqueous Polyaminopropyl Biguanide	Groups of 110 mice (55 males, 55 females) of the C57Bl/10J/CD-1 Alpk strain.	4 groups received dietary concentrations of 0, 400, 1200, and 4000 ppm (0, ~55, ~167, and ~715 mg/kg/d, respectively) for 2 years	Mortalities increased in the 4000-ppm group; hemangiosarcoma was most frequent factor causing death. At 4000 ppm, increases in squamous cell carcinomas of the recto-anal junction (5 males and 8 females); also, in 1 male, 1 adenocarcinoma at same site and a squamous cell carcinoma of the skin adjacent to the anus. Gall bladder papillomas in males at 4000 ppm. Highest incidence of treatment-related tumors at 4000 ppm was in neoplasms of vascular origin (ie, hemangiosarcomas, common tumor in C57Bl/10J/CD-1 Alpk mice). Hemangiosarcoma and hemangioma incidences (in liver and other sites) at 4000 ppm were above control incidence; findings statistically significant in male mice only. Small increased incidence of hemangiosarcomas in 1200 ppm group. Some evidence of carcinogenicity.[18]
20.2% aqueous Polyaminopropyl Biguanide	Wistar rats (20 males, 20 females)	Oral dosage rates 100 mg/kg/d for 25 months.	No findings of clinically apparent tumors. Testicular tumor in 1 male. Mammary tumor (benign adenofibroma) in 1 female. Classified as inadequate study for various reasons, including that only 20 rats per sex, no controls, and only 1 dose tested.[21]
Polyaminopropyl Biguanide	Groups of Wistar-derived Alpk: ApfSD rats	Concentrations of 0, 200, 600, or 2000 ppm (approximately equivalent to 0, 12.1, 36.3, and 126.1 mg/kg/d in males and 0, 14.9, 45.3, and 162.3 mg/kg/d in females) in diet for 2 years.	Hemangioma (2/64 males and 2/64 females) and hemangiosarcoma (1/64 females) in the liver of one animal fed 2000 ppm.[30]
20.2% aqueous Polyaminopropyl Biguanide	Groups of 60 male and 60 female rats of unspecified strain	4 groups fed at concentrations of 0, 200, 1000, and 2000 ppm for 124 weeks	Study terminated at 124 weeks, due to 80% mortality. 2 outbreaks of infection noted. Long-term exposure unrelated to carcinogenic and other effects. Hemangiomas at week 52 in 1/12 male rats (mesenteric lymph nodes) fed 200 ppm and 1/12 male rats fed 200 ppm (cervical lymph nodes). Hemangiomas at week 104 in 2/12 males fed 1000 ppm (mesenteric lymph nodes) and in 1/8 females fed 200 ppm (uterus). Hemangiosarcoma at week 104 in 1/21 males fed 2000 ppm (mesenteric lymph nodes). Hemangiomas at week 124 (end of study) in 1/20 males fed 1000 ppm (mesenteric lymph nodes) and in 1/19 males fed 2000 ppm (spleen). No vascular tumors in controls. Study of questionable reliability due to infections and < 50% survival at end of study.[18]

(continued)

Table 12. (continued)

Ingredient	Animals/Cells	Protocol	Results
Polyaminopropyl Biguanide (in drinking water or in the diet)	Groups of rats (strain and number per group not stated).	In a 2-year study, Polyaminopropyl Biguanide (in drinking water) administered to groups of rats (strain and number per group not stated) at concentrations of 500 mg/L, 1000 mg/L, and 1500 mg/L. Test substance also administered (in diet) to group of rats at a dose of 4000 mg/kg.	Liver identified as main target organ for Polyaminopropyl Biguanide. Hepatocellular tumors induced at concentrations of 1000 mg/L and 1500 mg/L, but not at a concentration of 500 mg/L. Tumor incidence greater in male rats than in female rats. Tumors had prominent vascular changes (changes in test and control rats) that resulted in initial misclassification as vascular tumors. Vascular changes in tumors later classified as ectasia. All tumors reclassified as hepatocellular foci, adenomas, and carcinomas. Administration of Polyaminopropyl Biguanide in diet did not cause increase in hepatocellular tumors.[20]
			(The experiments that follow were performed to determine the mode of action [MOA] of Polyaminopropyl Biguanide.)
Polyaminopropyl Biguanide (in drinking water and in diet)	Groups of 10 male Wistar Han [Crl: WI(Han)] rats	Three groups received Polyaminopropyl Biguanide (in drinking water) at doses of 500, 1000, and 1500 mg/L, respectively ($\sim$ 50, 100, and 150 mg/kg, respectively) for 4 weeks. A fourth group received Polyaminopropyl Biguanide (in powdered diet) at a dose of 4000 mg/kg for 4 weeks, and another group served as the control (no test substance exposure). Animals were killed at end of study and necropsied. Liver sections prepared for histopathology and immunohistochemistry was performed on additional liver sections and a section of the duodenum. To determine the mode of action (MOA) for liver tumor induction (ie, in 2 year carcinogenicity study above; tumor formation in preceding carcinogenicity study) observed in, the potential for increased proliferation of hepatocytes and endothelial cells in the liver evaluated using endogenous marker for DNA synthesis (Ki-67). Double-labeling method identifying endothelial cells (CD-31) utilized. Monoclonal mouse anti-rat CD31 antigen and monoclonal anti-rat-Ki-67 were used. Endothelial cell proliferation determined by the number of Ki-67 positive cells in $\sim$ 1000 CD-31 positive cells in liver tissue.	Dose responsive increase in endothelial cell labeling index. Increase was statistically significant in 150 mg/kg dose (in drinking water) group and in the 4000 mg/kg dose (in diet) group.[20]
Polyaminopropyl Biguanide (in drinking water and in diet)	Groups of 10 male Wistar Han [Crl: WI(Han)] rats Same test groups, as defined above.	Another experiment relating to determination of MOA. Effect on serum and liver TNFα, NF$\kappa\beta$, and IL-1α, (mitogenic cytokines) evaluated	Polyaminopropyl Biguanide (in drinking water) caused a statistically significant decrease ($P < 0.05$) in the serum concentration of IL-1α, at a dose of 150 mg/kg. The same was true for Polyaminopropyl Biguanide at a dietary dose of 4000 mg/kg. Polyaminopropyl Biguanide (in drinking water or in diet) had no effect on the serum or liver TNFα and NF$\kappa\beta$ levels. Based on results from the short-term oral toxicity study (summarized in Table 9), the metabolism study (in ADME section) and these 2 MOA experiments on Polyaminopropyl Biguanide (all by same authors), the hypothesized MOA was determined to be: severe dehydration and starvation because of unpalatability, followed by ingestion with rapid absorption and urinary excretion; increased hepatocyte proliferation; and induction of hepatocellular foci and tumors.[20]

Abbreviations: ADME, Absorption, Distribution, Metabolism, and Excretion; NOAEL, no observed adverse effect level; SCCS, Scientific Committee on Consumer Safety; SPF, specific pathogen free.

Table 13. Dermal Irritation and Sensitization Studies.

Ingredient	Number of Animals/Subjects	Protocol	Results
		Irritation Studies	
Animal Studies			
Polyaminopropyl Biguanide (0.2% in ethanol, 10% in ethanol, and 20% [solvent not specified])	4 groups of SPF Alderley Park mice (50 males, 50 females)	Test substance (0.3 mL) was administered at the following doses 5 days per week for 80 weeks: 0 mg/d (in ethanol), 0.6 mg/d (0.2% Polyaminopropyl Biguanide in ethanol), 6.0 mg/d (20% Polyaminopropyl Biguanide and 30 mg/d [10% Polyaminopropyl Biguanide] in ethanol).	The highest dose (10% concentration; 30 mg/d) caused noticeable skin irritation in males and females immediately after application. Erythema observed during first few weeks. After 4th week, hyperkeratosis became evident, especially in males. Also, occasionally, there was ulceration extending to the deeper layers of the dermis at the application site.[21]
20.2% aqueous Polyaminopropyl Biguanide	Groups of 10 rats (5 males, 5 females per group) of the Alpk: APfSD (Wistar-derived) strain	3 groups received applications (occlusive, on the back) of the test substance at doses of 20 mg/kg/d, 60 mg/kg/d, and 200 mg/kg/d, respectively, 6 hours per day for 30 days (21 applications total).	Slight irritation at 60 mg/kg/d; in most animals, had regressed by end of study. Moderate irritation in all animals at 200 mg/kg/d; in most animals, persisted until end of study. Skin irritation observed was confirmed microscopically and considered test substance-related.[18]
20% aqueous Polyaminopropyl Biguanide	5 female rats of the Alderley Park strain	Test substance (0.04% active ingredient) applied (0.1 mL; mg/cm^2 not stated) to the back on alternate days (6 applications total). Test site remained uncovered or was covered with polyethylene, secured with an adhesive plaster, for 24 hours.	Nonirritant.[21]
25% aqueous Polyaminopropyl Biguanide	2 groups of 20 (10 males, 10 females/group) healthy SPF albino rats	2 groups received a topical application of test substance to intact skin at dosages of 2.5 mL/kg and 5 mL/kg, respectively. Test substance spread over 1 in^2; site covered with dressing for 24 hours.	Severe skin irritation in all animals.[21]
25% aqueous Polyaminopropyl Biguanide	3 female rats (strain not stated)	Test substance applied (dose not specified) under occlusive dressing to intact skin of back for 3 alternating 24-hour periods, ie, each application period followed by 24-hour nontreatment period.	Focal ulceration observed after first 24-hour application. Reaction increased in severity after 2nd and 3rd applications, by which time there was pronounced edema.[21]
25% aqueous Polyaminopropyl Biguanide	Albino guinea pigs (6 test and 4 control) of Porton strain	Both ears treated (patch application; 0.1 mL per ear) with 25% Polyaminopropyl Biguanide once per day for 3 consecutive days. Next, 0.2 mL of following concentrations (in dimethylformamide) applied to flank (1 cm^2): 2510%, 12.5%, and 1025%	Slight to moderate erythema (irritant effect) on ear at 25%.[18]
20% aqueous Polyaminopropyl Biguanide	6 female albino rabbits	12,000 ppm solution (1 mL) applied to back for 23 hours (m/v not stated; no occlusion). 21 daily applications.	Nonirritant.[18]
Polyaminopropyl Biguanide	5 male New Zealand White rabbits	Test substance (0.5 g, moistened with distilled water) applied to 3 sites on back (mg/cm^2 not stated); sites covered with cotton gauze patch secured with adhesive tape. Patches removed after 3 minutes, 1 hour, or 4 hours.	Slight edema at 1 hour after patch removal and very slight edema at 24 hours and 48 hours. After 4 hours, very slight to well-defined erythema; primary irritation index (PII) = 1. Mean values (at 24 hours, 48 hours, and 72 hours) for erythema, eschar formation or edema formation calculated for each animal tested were $\leq$ 1. No skin reactions after 7 days. Mild skin irritant.[18]

(continued)

Table 13. (continued)

Ingredient	Number of Animals/Subjects	Protocol	Results
Polyaminopropyl Biguanide (96%, as powder)	3 male rabbits (strain not specified)	Test substance (0.5 g moistened with 0.5 mL water) applied under occlusive patch to 3 sites on back of 1 rabbit; mg/cm^2 not stated. Patches removed after 3 minutes, 1 hour, or 4 hours. For remaining 2 rabbits, patch remained in place for 4 hours.	No irritation after 3-minute or 1-hour application. After 4-hour exposure, primary irritation index of 1 reported; very slight (at 1 hour, 48 hours, and 72 hours after patch removal) to well-defined (at 4 hours and 24 hours) erythema observed. Slight edema (at 1 hour) and very slight edema (at 24 hours and 48 hours). No reactions at 7 days after patch removal. Mild skin irritant.[3]
20% aqueous Polyaminopropyl Biguanide	9 (3 males, 6 females) New Zealand White rabbits	Test substance applied to 6 rabbits (0.5 mL, under occlusive dressing) for 24 hours to $\sim$ 6.25 cm^2 of intact and abraded skin of the flanks. Similar application to 3 male rabbits; animals then killed at 48 hours or 72 hours post-application for histopathologic examination of test site.	Moderately irritating to intact skin. Severely irritating to abraded skin.[18]
20% aqueous Polyaminopropyl Biguanide	3 rabbits (strain not specified)	Applied to skin for 24 hours (m/v not stated).	Moderate erythema at 24 hours post-application. Completely reversible within 8 days. No edema.[18]
20% aqueous Polyaminopropyl Biguanide	6 New Zealand White rabbits	Skin corrosivity test. Applied to intact and abraded skin (n/v and duration of application not stated).	Superficial scabbing and erythema around the abrasions. No signs of necrosis at intact skin sites. Noncorrosive.[18]
Human Studies			
20% aqueous Polyaminopropyl Biguanide	45 volunteers (17 males, 28 females)	Following concentrations (in purified water) applied topically (Finn chamber) for 24 hours to medial surface of upper arm: 0.3%, 0.6%, and 1.5% active ingredient.	Plaster dermatitis observed in all test groups, including vehicle controls. Skin irritation indices of 6.6, 5.5, 5.5, and 8.8 obtained for concentrations of 0 (vehicle control), 0.3, 0.6, and 1.5% active ingredient. Not a primary skin irritant, given the similarity of skin irritation indices between test and control groups.[18]
Bacterial nanocellulose dressing loaded with 1% wt/vol sericin and 0.3% wt/vol Polyaminopropyl Biguanide	105 healthy volunteers	Initially, skin randomly patched with dressings (2 cm $\times$ 2 cm). After 3 days, new dressings patched onto same area. After an additional 3 days, dressings removed; removal followed by 7- to 10-day nontreatment period. Skin then patched (open and closed patch tests) with dressings on same area. After 3 days, dressings removed.	Majority of test sites did not show edema (more than 98%) or papules (more than 97%). Neither vesicles nor bullae were observed on the skin. Dressing classified as nonirritating to the skin.[34]
Sensitization Studies			
Animal Studies			
Polyaminopropyl Biguanide		Local lymph node assay (protocol details not provided). Positive results defined as one or more test concentrations eliciting a 3-fold or greater increase in proliferative activity, compared with concurrent vehicle control	Positive results.[35,36]

(continued)

Table 13. (continued)

Ingredient	Number of Animals/Subjects	Protocol	Results
20% aqueous Polyaminopropyl Biguanide	10 Alderley Park guinea pigs (test animals) and 10 control guinea pigs.	Buehler test. Concentration of 10% (2% active ingredient, 0.4 mL) applied to scapular region (400 mm^2) during topical induction (occlusive dressing) for 6 hours. Induction repeated 3 times/week for 3 weeks (10 applications total). Challenge exposures (2% active ingredient) of 6 hours performed 2 weeks after last induction exposure. Rechallenge with concentrations of 20%, 10%, and 1% (4%, 2%, and 0.2% active ingredient, respectively).	Faint erythema in 6 of 10 test animals. Rechallenge yielded faint erythema at concentrations of 4% (8 of 9 animals) and 2% (3 of 10 animals) active ingredient. No reaction to 0.2% active ingredient. 2% active ingredient considered moderate sensitizer (classification scheme not stated).[18]
20% aqueous Polyaminopropyl Biguanide	Groups of 20 (10 males and 10 females per group) guinea pigs	Buehler test. Induction and challenge concentrations: induction (0.3%) and challenge (0.3%, 0.15%, 0.075%, and 0.03%); induction (0.8%) and challenge (0.8%, 0.4%, 0.2%, and 0.08%); induction (1.3%) and challenge (1.3%, 0.65%, 0.325%, and 0.13%); induction (1.8%) and challenge (1.8%, 0.9%, 0.45%, and 0.18%); induction (2%), challenge (2%), and rechallenge (2%); 1.2% induction, challenge (1.2%), and rechallenge (1.2% and 15%); and induction (5%), challenge (15%), and rechallenge (2% and 1.2%).	Threshold for eliciting sensitization in guinea pigs was approximately 1%.[18]
20% aqueous Polyaminopropyl Biguanide (diluted with saline)	Groups of 10 guinea pigs	Guinea pig maximization test. Intradermal induction with 0.15% Polyaminopropyl Biguanide and topical induction with 20%. Challenge with 20% or 10%.	Moderate erythema at 10% and 20% (1 of 10 animals per concentration). Nonsensitizer (classification scheme not stated).[18]
20% aqueous Polyaminopropyl Biguanide	20 Alderley Park female guinea pigs (test animals) and 8 female guinea pigs (controls)	Guinea pig maximization test. Intradermal induction (in scapular region) with 1% of test substance as delivered (0.2% active ingredient). Topical induction and challenge with 20.2% active ingredient.	Mild to moderate erythema in 14 of 20 animals (at 24 hours) and in 15 of 20 animals (at 48 hours). Moderate to strong sensitizer (classification scheme not stated).[18]
20% aqueous Polyaminopropyl Biguanide	Female Dunkin Hartley guinea pigs (20 test and 8 control animals).	Guinea pig maximization test. Possible cross-reactivity with chlorhexidine also evaluated. Intradermal induction with 0.25%. Topical induction and challenge with 20% Polyaminopropyl Biguanide. Challenge with 0.05%, 0.5%, and 4% chlorohexidine gluconate.	Challenge reactions to 20% in 8 of 20 animals. Reactions in 3 of 20 at rechallenge. No cross-reactivity with chlorhexidine. Test substance was mild sensitizer (classification scheme not stated).[18]
20.2% aqueous Polyaminopropyl Biguanide	20 female Alpk: Dunkin Hartley guinea pigs (test group) and 10 female guinea pigs (control group).	Guinea pig maximization test. Induction phase: intradermal induction (0.3% of test substance as delivered [0.06% active ingredient], 0.1 mL in shoulder region). One week later, dermal induction performed by occlusively applying neat substance (20.2% active ingredient) to induction sites for 48 hours. Challenge: occlusive epicutaneous application (24 hours) of undiluted test substance (20.2% active ingredient) and a 30% solution in deionized water (6% active ingredient) to previously untreated site.	Scattered mild redness or moderate diffuse redness observed in 18/20 test animals at 24 hours and 16/20 test animals at 48 hours. Moderate sensitizer (classification scheme not stated).[18]

(continued)

Table 13. (continued)

Ingredient	Number of Animals/Subjects	Protocol	Results
Human Studies			
20% aqueous Polyaminopropyl Biguanide	191 subjects (49 on Panel 1, 114 on Panel 2, and 28 on Panel 3)	During induction, test substance applied (2 cm × 2 cm patches moistened with 0.5 mL aliquots) for 24 hours to dorsal surface of upper arm at concentrations of 2% active ingredient (effective concentration= 0.4%) and 4% (effective concentration = 0.8%). Repeated 3 times per week for 10 applications total. Applied at following concentrations during challenge phase: 0.05% (effective concentration = 0.01%), 0.1% (effective concentration = 0.02%), 0.2% (effective concentration =0.04%), 0.5% (effective concentration = 0.1%), 1% (effective concentration = 0.2%), and 2% (effective concentration = 0.4%).	Panel 1: At challenge, 8 of 49 subjects (16%) had skin reactions to 0.4%, 7 of 49 (14%) with reactions to 0.2% and 0.1%, and 2 of 49 (4%) with weak reactions at 0.02%. Panel 2: 18 of 114 subjects (16%) with skin reactions to 0.1% and 7 of 114 (6%) with reactions to 0.04%. 2 other subjects with reactions during nontreatment period following 0.4% induction, characterized as likely allergic to 0.4%. The same was true for 10 other subjects regarding reactions (described as weak) at late 0.4% induction. Panel 3: 1 of 28 subjects (3.6%) with reaction to 0.1%. Conclusion: 0.4% concentration not capable of causing primary skin irritation, but capable of causing skin sensitization humans.[3]
Leave-on product containing 20% Polyaminopropyl Biguanide (tested at 0.5%; effective test concentration = 0.1% Polyaminopropyl Biguanide)	207 subjects	In HRIPT, product (0.1 g on a 2 cm × 2 cm occlusive patch) applied to skin (48-hour to 72-hour application) at dose density of 25 mg/cm^2. Dose density of Polyaminopropyl Biguanide applied to skin calculated to be 0.025 mg/cm^2 (25 μg/cm^2). 3-week induction period followed by 2-week nontreatment period. Challenge patch applied to a new test site. Reactions scored at 24 hours, 48 hours, 72 hours, and 96 hours.	Product did not induce dermal sensitization.[37]
Neck cream containing 0.2% Polyaminopropyl Biguanide	115 male and female subjects (58 African Americans, 43 Caucasians, and 13 Hispanics). Subjects were free of any systemic or dermatological disorder that might have interfered with the study. Also, subjects were of any skin type or ethnicity, provided that their degree of skin pigmentation did not significantly interfere with evaluations.	During induction, the product was applied (2 cm × 2 cm occlusive patches containing 0.2 mL of product) for 24 hours to upper back (dose = 100 μg/cm^2); this was repeated 3 times per week for 3 weeks. Challenge patch applied for 24 hours to new site on opposite side of upper back.	Transient, barely perceptible to mild erythema in 43 of 115 subjects (37% of subjects tested) during induction and/or challenge phases: 34 Caucasians, 6 Hispanics, and 3 African Americans. No evidence of clinically meaningful irritation, and no reactions allergic in nature.[38]
20% Polyaminopropyl Biguanide solution, diluted with distilled water to 1% vol/vol prior to testing (dose = 750 μg/cm^2)	108 subjects Asian (∼ 2%), Biracial (∼ 3%), Black (∼ 23%), Caucasian (∼ 33%), and Hispanic (∼ 39%). Data on Fitzpatrick skin types are not included.	HRIPT. During induction, ∼ 2 cm × 2 cm semi-occlusive patches containing 0.3 mL of the test material were applied for ∼ 24 hours to left side of back, 3×/week for 3 weeks, for a total of 9 applications. Test sites were score 24-hour (weekdays) or ∼ 48-hour (weekends) after patch removals. Challenge phase initiated after ∼ 2-week nontreatment period. ∼ 2 cm × 2 cm semi-occlusive patch containing 0.3 mL the test material applied for ∼ 24 hours to new site on opposite side of back. Using modified scoring scale of International Contact Dermatitis Research Group, reactions were scored at ∼ 24 hours, ∼ 48 hours, ∼ 72 hours, and ∼ 96 hours after patch application. Patch testing with vehicle (distilled water) and saline controls according to same procedure.	During induction, low-level (±) reactions observed in 2 subjects. Reactions not observed during challenge phase. Authors noted that no adverse reactions or adverse events reported or observed in any of the subjects tested, and concluded that Polyaminopropyl Biguanide did not induce dermal sensitization in the subjects tested. When the 108 subjects were patch-tested with the vehicle control (distilled water) according to the same procedure, low-level (±) reactions were observed in 2 subjects during induction, and in 4 subjects during the challenge phase. Additionally, when the 108 subjects were patch-tested with saline (control), low-level (±) reactions were observed in 2 subjects during induction, and in 1 subject during the challenge phase. The authors concluded that neither control induced dermal sensitization in the subjects tested.[39]

(continued)

Table 13. (continued)

Ingredient	Number of Animals/Subjects	Protocol	Results
		Patients	
20% aqueous Polyaminopropyl Biguanide	1975 patients	Multicenter study. Patch testing with 2.5% aqueous (effective concentration = 2.5% × 20% = 0.5%) and 5% aqueous (effective concentration = 5% × 20% = 1%). Frequencies of sensitization (as % of patients tested) calculated as crude proportions and additionally standardized for sex and age.	10 patients (0.5%) with positive reaction and 16 patients (0.8%) with positive reaction to1%. Assumed that, probably, at least 4 reactions at to 0.5% may have been doubtful or irritant, ie, false positive, because were not confirmed by simultaneous reactions to higher concentrations. Probable cause of sensitization was occupational exposure. Other risk factors included leg dermatitis and old age.[48]
2.5% aqueous Polyaminopropyl Biguanide	374 patients (multicenter study in United Kingdom)	Patch test (protocol not described)	2 positive patch test reactions. Data series suggested that baseline frequency of Polyaminopropyl Biguanide sensitization was very low (0.5%) in United Kingdom. Majority of reactions were weak, and data suggested that Polyaminopropyl Biguanide may not be a relevant contact allergen.[46,45]
20% aqueous Polyaminopropyl Biguanide	1554 male and female patients	Multicenter study. Patch tests (performed in accordance with recommendations of the International Contact Dermatitis Research Group [ICDRG] and the German Contact Dermatitis Research Group [DKG]) on 2.5% aqueous test substance (effective concentration = 2.5% × 20% = 0.5%). Applied to 389 patients for 1 day and to 1165 patients for 2 days.	6 patients (0.4%) with positive (+) reaction. One of the reactions in patient with atopic dermatitis may have been a false positive. Polyaminopropyl Biguanide sensitization considered extremely rare.[47]

Abbreviations: HRIPT, human repeated insult patch test; SPF, specific pathogen free.

Table 14. Quantitative Risk Assessment (QRA) Worksheet, Utilizing a NESIL of 1000 μg/cm^2.[40]

Product category	Max use (%)	Product exposure (μg/cm^2)	PHMB CEL (μg/cm^2)	PHMB NESIL (μg/cm^2)	SAF	PHMB AEL	AEL/ CEL	Reference for exposure
Baby lotions, oils, powders, creams	0.1	2200	2.20	1000	300	3.33	1.52	IFRA RIFM QRA Information Booklet.[64]
Eye shadow	0.03	2170	0.65	1000	300	3.33	5.12	Eye products.[65]
Eye lotion	0.2	2170	4.34	1000	300	3.33	0.77	Eye products.[65]
Eye makeup remover	0.056	900	0.50	1000	100	10.00	19.84	Make-up remover.[65]
Mascara	0.1	2170	2.17	1000	300	3.33	1.54	Eye products.[65]
Other eye makeup	0.01	2170	0.22	1000	300	3.33	15.36	Eye products.[65]
Hair conditioners	0.06	200	0.12	1000	100	10.00	83.33	Conditioners, rinse-off.[65]
Hair straighteners	0.01	4200	0.42	1000	100	10.00	23.81	IFRA RIFM QRA Information Booklet.[64]
Shampoos (noncoloring)	0.008	170	0.01	1000	100	10.00	735.29	Shampoos.[65]
Tonics, dressings and other hair grooming aids	0.1	990	0.99	1000	100	10.00	10.10	Hair styling aids.[65]
Other noncoloring hair products	0.002	1000	0.02	1000	100	10.00	500.00	IFRA/RIFM QRA Information Booklet.[64]
*Hair dyes and colors	0.1	1000	1.00	1000	100	10.00	10.00	IFRA/RIFM QRA Information Booklet.[64]
Foundations	0.01	3170	0.32	1000	100	10.00	31.55	Women's facial liquid makeup.[65]
Deodorants (underarm)	0.003	8500	0.26	1000	300	3.33	13.07	Solid AP.[65]
Other personal cleanliness products	0.006	4400	0.26	1000	300	3.33	12.63	Api et al., 2008 Intimate wipes.[65]
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	0.1	900	0.90	1000	100	10.00	11.11	Api et al., 2008 Make-up remover. [65]
Face and neck creams, lotions, powders, and sprays	0.02	2700	0.54	1000	100	10.00	18.52	Women's facial cream.[65]
Body and hand creams, lotions, and powders	0.009	1120	0.10	1000	300	3.33	33.07	Body creams and lotions.[65]
Moisturizers	0.00075	2700	0.02	1000	100	10.00	493.83	Women's facial cream.[65]
Skin fresheners	0.0085	150	0.01	1000	100	10.00	784.31	Face washes, gels, scrubs.[65]
Suntan gels, creams, liquids	0.1	2200	2.20	1000	100	10.00	4.55	IFRA RIFM QRA Information Booklet.[64]
Eye lotion with maximum supportable PHMB	0.15	2170	3.26	1000	300	3.33	1.02	Eye products.[65]

Abbreviations: AEL, acceptable exposure level; CEL, consumer exposure level; NESIL = no expected sensitization induction level; PHMB, polyhexamethylene biguanide; SAF, sensitization assessment factors.

Table 15. Quantitative Risk Assessment (QRA) Worksheet, Utilizing a NESIL of 750 μg/cm^2.[41]

Product category	Max use (%)	Product exposure (μg/cm^2)	PHMB CEL (μg/cm^2)	PHMB NESIL (μg/cm^2)	SAF	PHMB AEL	AEL/ CEL	Reference for exposure
Baby lotions, oils, powders, creams	0.1	2200	2.20	750	300	2.50	1.14	IFRA RIFM QRA Information Booklet.[64]
Eye shadow	0.03	2170	0.65	750	300	2.50	3.84	Eye products. [65]
Eye lotion	0.2	2170	4.34	750	300	2.50	0.58	Eye products. [65]
Eye makeup remover	0.056	900	0.50	750	100	7.50	14.88	Make-up remover.[65]
Mascara	0.1	2170	2.17	750	300	2.50	1.15	Eye products.[65]
Other eye makeup	0.01	2170	0.22	750	300	2.50	11.52	Eye products.[65]
Hair conditioners	0.06	200	0.12	750	100	7.50	62.50	Conditioners, rinse-off.[65]
Hair straighteners	0.01	4200	0.42	750	100	7.50	17.86	IFRA RIFM QRA Information Booklet.[64]
Shampoos (noncoloring)	0.008	170	0.01	750	100	7.50	551.47	Shampoos.[65]

(continued)

Table 15. (continued)

Product category	Max use (%)	Product exposure (μg/cm^2)	PHMB CEL (μg/cm^2)	PHMB NESIL (μg/cm^2)	SAF	PHMB AEL	AEL/ CEL	Reference for exposure
Tonics, dressings and other hair grooming aids	0.1	990	0.99	750	100	7.50	7.58	Hair styling aids.[65]
Other noncoloring hair products	0.002	1000	0.02	750	100	7.50	375.0	IFRA RIFM QRA Information Booklet.[64]
*Hair dyes and colors	0.1	1000	1.00	750	100	7.50	7.50	IFRA RIFM QRA Information Booklet.[64]
Foundations	0.01	3170	0.32	750	100	7.50	23.66	Women's facial liquid makeup.[65]
Deodorants (underarm)	0.003	8500	0.26	750	300	2.50	9.80	Solid AP.[65]
Other personal cleanliness products	0.006	4400	0.26	750	300	2.50	9.47	Intimate wipes.[65]
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	0.1	900	0.90	750	100	7.50	8.33	Make-up remover.[65]
Face and neck creams, lotions, powders, and sprays	0.02	2700	0.54	750	100	7.50	13.89	Women's facial cream.[65]
Body and hand creams, lotions, and powders	0.009	1120	0.10	750	300	2.50	24.80	Body creams and lotions.[65]
Moisturizers	0.00075	2700	0.02	750	100	7.50	370.37	Women's facial cream.[65]
Skin fresheners	0.0085	150	0.01	750	100	7.50	588.24	Face washes, gels, scrubs.[65]
Suntan gels, creams, liquids	0.1	2200	2.20	750	100	7.50	3.41	IFRA RIFM QRA Information Booklet.[64]
Eye lotion with maximum supportable PHMB	0.11	2170	2.39	750	300	2.50	1.05	Eye products.[65]

Abbreviations: AEL, acceptable exposure level; CEL, consumer exposure level; NESIL, no expected sensitization induction level; PHMB, polyhexamethylene biguanide; SAF, sensitization assessment factors.

Table 16. Ocular Irritation Studies.

Ingredient	Number of Animals	Test Protocol	Results
		In Vitro Study	
20% aqueous Polyaminopropyl Biguanide	Donated human eyes (41) and rabbit eyes	Applied (20 μL for 10 seconds; 100 μL for 1 minute) at superior limbus. Eyes situated in temperature-controlled chamber during application.	1-minute exposure did not cause change in corneal thickness. Normal corneal morphology at histological examination.[43]
		Animal Studies	
20% Polyaminopropyl Biguanide (diluted to 0.04% active ingredient)	3 rabbits (strain not stated)	Test substance (0.1 mL) instilled into eyes	No immediate or delayed irritant effects observed.[21]
20% aqueous Polyaminopropyl Biguanide	9 female New Zealand White rabbits	Test substance (0.1 mL) instilled into conjunctival sac of 1 eye; contralateral eye served as untreated control. Eyes of 6 animals not rinsed after instillation. Eyes of remaining 3 animals were rinsed.	Iritis and conjunctivitis in unrinsed eyes and 4/6 rabbits with transient corneal opacity. Conjunctivitis, but no corneal reaction, in rinsed eyes and slight iritis in 1 rabbit. Test substance was moderate eye irritant in unrinsed eyes and a mild irritant in rinsed eyes.[3]
20% Polyaminopropyl Biguanide	3 rabbits (strain not stated)	Test substance (0.12 mL) instilled into 1 eye, followed by rinsing with saline	Slight inflammation, but no corneal ulceration. Changes resolved in 10 days.[21]
25% aqueous Polyaminopropyl Biguanide	3 rabbits (strain not specified). 3 rabbits (strain not specified). 3 rabbits (strain not specified).	Single instillation (volume not specified). Procedure repeated with saline rinse after instillation	Severe inflammation and corneal damage in all rabbits (unrinsed eyes). Condition partly resolved in 2 rabbits. 3rd rabbit blinded in treated eye. In rinsed eyes, only slight inflammation observed; eyes normal by day 3.[21]

(continued)

Table 16. (continued)

Ingredient	Number of Animals	Test Protocol	Results
Polyaminopropyl Biguanide (powder form, 99.6% pure)	1 New Zealand rabbit	Test substance (0.1 g) instilled into 1 eye.	Moderate redness, chemosis, moderate corneal opacity, iridial congestion, and ulceration of the nictitating membrane and cornea. Severe ocular irritant.[3]
Polyaminopropyl Biguanide (undiluted)	1 male New Zealand White rabbit	Test substance (0.1 mL) instilled into conjunctival sac of right eye; untreated eye served as control. Eye not rinsed after instillation.	Opalescent corneal opacity, iridial inflammation, and severe conjunctival irritation observed initially. Translucent corneal opacity, minimal conjunctival irritation, and vascularization were noted at day 21 postinstillation and considered irreversible reactions. Test substance was corrosive to rabbit eye.[3]

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