Safety Assessment of Polyaminopropyl Biguanide as Used in Cosmetics

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date to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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

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INTRODUCTION

The safety of Polyaminopropyl Biguanide as used as a preservative in cosmetics is reviewed in this assessment. The ingredient name, Polyaminopropyl Biguanide, refers to an amino polymer comprising propyl biguanide repeat units (polyaminopropyl biguanide (PABA)) ***or*** hexyl biguanide repeat units (polyhexamethylene biguanide (PHMB)), ***or*** hydrochloride salts thereof. Thereby, the ingredient name, Polyaminopropyl Biguanide, represents four similar, but distinct chemicals. Accordingly, throughout this report, the ingredient name Polyhexamethylene Biguanide will be used exclusively (as only one ***ingredient*** is under review), and when the chemical under consideration is other than PABA, a notation will be made (e.g., "Polyaminopropyl Biguanide (as PHMB HCl) was tested…"). In 2016, the SCCS issued a revised opinion (preliminary opinion) stating that the use of Polyaminopropyl Biguanide as a preservative in all cosmetic products at concentrations up to 0.1% is safe. The opinion also states that, because no new safety data on inhalation are available on Polyaminopropyl Biguanide, its use in sprayable formulations is not advised. The comment period on this preliminary opinion ends on March 10, 2017.[1]

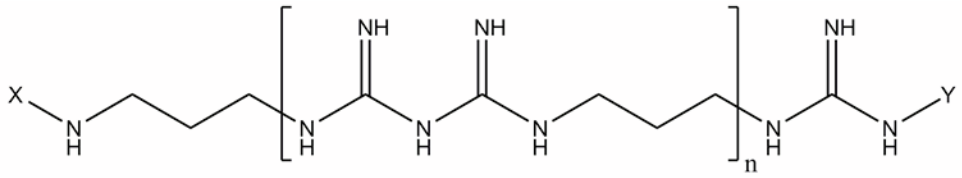
Additionally, the safety of the hydrochloride salt of polyhexamethylene biguanide, one of the four chemicals referred to as Polyaminopropyl Biguanide, has been reviewed by the United States Environmental Protection Agency (EPA), and the Agency concluded that its use as a pesticide has very low aggregate risk of adverse health effects to the public or environment.[2]

The majority of the information (published and unpublished data) identified for inclusion in this safety assessment are on the hydrochloride salt of polyhexamethylene biguanide, one of the four chemicals refered to as Polyaminopropyl Biguanide.

CHEMISTRY

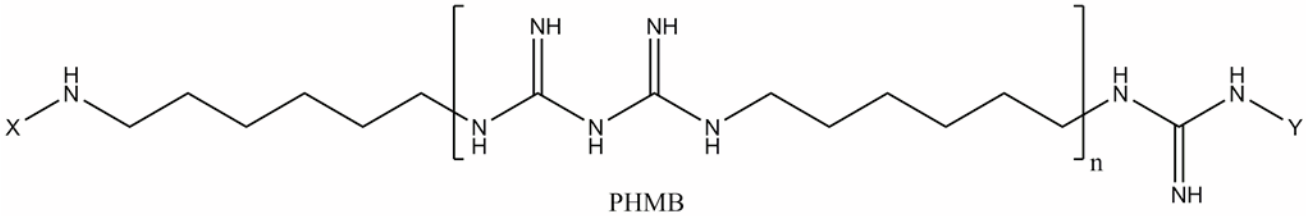
Definition and General Characterization

Polyaminopropyl Biguanide is an amino polymer comprising propyl biguanide repeat units (polyaminopropyl biguanide (PABA)) ***or*** hexyl biguanide repeat units (polyhexamethylene biguanide (PHMB)), ***or*** hydrochloride salts thereof, is stated in Table 1.[3]



PABA

or



PHMB

wherein X may be hydrogen or the hydrochloride salt, and Y may be nitrile or the hydrochloride salt.

Figure 1. The cosmetic ingredient name, Polyaminopropyl Biguanide, is used for both PABA and PHMB.

However, the current wINCI monograph for Polyaminopropyl Biguanide is not completely clear. There is no mention of PHMB in the name or definition. However, a structure is provided for the hydrochloride salt of PHMB (PHMB HCl), one of the CAS Nos. also refers to PHMB HCl, and the other CAS number refers to PABA. As there is no separate ingredient INCI name for PHMB, the Scientific Committee on Consumer Safety (SCCS) review of Polyaminopropyl Biguanide also included PHMB HCl.[4] Accordingly, throughout this report, the ingredient name Polyhexamethylene Biguanide will be used exclusively (as only one ***ingredient*** is under review), and when the chemical under consideration is other than PABA, a notation will be made (e.g., "Polyaminopropyl Biguanide (as PHMB HCl) was tested…").

Chemical and Physical Properties

Polyaminopropyl Biguanideis a polymer that, in its neat form, is a solid/powder with purity > 94.2 %, and is often marketed as an approximately 20% aqueous solution.[4] Chemical and physical properties are summarized in Table 2.

Method of Manufacture

One of the current methods for manufacturing Polyaminopropyl Biguanide (as PHMB HCl) is via the polycondensation of sodium dicyanamide and hexamethylenediamine.[5]

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-amnohexyl)guanidine), *N*-cyano-*N*'-6-(6-guanidinohexyl)guanidine hydrochloride, and 1,6-diguanidinohexane dihydrochloride.[1]

The trace metals content (in ppm, w/w) of 5 different batches of Polyaminopropyl Biguanide 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).[1]

USE

Cosmetic

The safety of Polyaminopropyl Biguanide is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database.[6] Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.[7]

According to 2016 VCRP data, Polyaminopropyl Biguanide is being used in 128 cosmetic products, mostly leave-on products.[6] The results of a concentration of use survey provided in 2016 indicate that Polyaminopropyl Biguanide is being used at concentrations up to 0.5% in both rinse-off and leave-on products (Table 3).[7]

Cosmetic products containing Polyaminopropyl Biguanide may be applied to the skin and hair or, incidentally, may come in contact with the eyes (at maximum use concentrations up to 0.3%) and mucous membranes. Additionally, Polyaminopropyl Biguanide is being used in a lipstick product, the application of which may result in incidental ingestion. 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.

Polyaminopropyl Biguanide is being used in both pump hair sprays (at 0.000002%-0.27%) and aerosol hair sprays (at 0.00025%-0.0004%) which may result in incidental ingredient inhalation exposure. 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.[8,9,10,11] Therefore, most

droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. [8,9]

The SCCS has concluded that Polyaminopropyl Biguanide is not safe for consumers when used as a preservative in all cosmetic products up to the maximum concentration of 0.3%.[4] In 2016, the SCCS issued a preliminary revised opinion stating that the use of Polyaminopropyl Biguanide as a preservative is safe in all cosmetic products at concentrations up to 0.1%. The opinion also states that, because no new safety data on inhalation are available on Polyaminopropyl Biguanide, its use in sprayable formulations is not advised. The comment period on this preliminary opinion ends on March 10, 2017.[1]

Polyaminopropyl Biguanide (CAS Nos. 133029-32-0 (PABA), 32289-58-0 (PHMB HCl), 27083-27-8 (PHMB HCl), and 28757-47-3 (PHMB)) is currently listed in Annex V (entry 28) of the European Commission (EC) Regulation No. 1223/2009 (Cosmetic Regulation) as a preservative to be used in all cosmetic products up to a maximum concentration of 0.3%.[1,13] Additionally, Polyaminopropyl Biguanide is classified as CMR 2 (Carc. 2) according to the Commission Regulation (EU) No. 944/2013. CMR substances are substances that are classified as carcinogenic, mutagenic, or toxic for reproduction in cosmetic products. 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 IA (substances known to have carcinogenic potential for humans) or Category IB (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, Polyaminopropyl Biguanide is considered prohibited as a cosmetic ingredient as of this date.[1] However, Article 15 (1) of the Cosmetics Regulation states that a substance classified in Category 2 may be used in cosmetic products if the substance has been evaluated by the SCCS and found safe for use in cosmetic products.

Polyaminopropyl Biguanide, a preservative, has been banned from personal care products in Denmark since January of 2015, based on the European Commission's classification of this ingredient as a CMR substance.[12] Reportedly, a representative of the Association of Danish Cosmetics, Toiletries, Soap and Detergent Industries (SPT) has stated that the organization does not find the suspected cancer-causing Polyaminopropyl Biguanide to be illegal, because CMR-substances may be used in cosmetic products if a risk assessment shows that the use of the substance is safe. Reference was made to the SCCS's conclusion relating to a safe level of Polyaminopropyl Biguanide.

Noncosmetic

Polyhexamethylene Biguanide has been reported to be the most frequently used antiseptic in traumatic and orthopedic surgery.[13] According to another source, Polyhexamethylene Biguanide (as PHMB HCl) 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 non-food contact surfaces.[2]

Polyhexamethylene Biguanide is a broad-spectrum antimicrobial agent used in a variety of products including contact lens cleaning solutions, skin disinfectant solutions, and wound dressings.[14] Solid wound dressings are composed of various synthetically or naturally derived materials, and typically contain added antimicrobials such as silver, bismuth, chlorhexidine, bacitracin, or Polyhexamethylene Biguanide. Wound dressings are regulated by FDA as Class 1 medical devices (i.e., 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.[15]

In Australia, Polyhexamethylene Biguanide is listed in the Poisons Standard – the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP) in Schedule 6.[16] 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 labelled with 'Poison'. According to this standard, Polyhexamethylene Biguanide can be used in preparations containing concentrations of 5% or less and when packed and labeled for therapeutic use.

TOXICOKINETIC STUDIES

Dermal Penetration

Dermal penetration data are presented in Table 4.

In Vitro

The highest concentration of Polyaminopropyl Biguanide (as PHMB HCl) that was applied in these studies was ~ 20%. In one studiy, 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. At concentrations of 0.4%, 1.4%, 5%, and 20% Polyaminopropyl Biguanide (as PHMB HCl), 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), and 20% (late phase) [^{14}C]-Polyaminopropyl Biguanide (as PHMB HCl), absorption rates (ng/cm^2/h) through rat whole skin were 131, 3695, and 11940, respectively. Another study involved the application of Polyaminopropyl Biguanide (as PHMB HCl) (5% solution) to rat skin biopsies of newborn hairless rats and human epidermal skin in diffusion chambers. For rat skin, no skin absorption was detected up to day 5 of exposure. For human epidermal skin biopsies, a low rate of penetration (~0.09 %) was noted after 24 h. Polyaminopropyl Biguanide (as PHMB HCl) (0.1% aqueous) was the lowest concentration applied (human split-thickness skin) in dermal penetration studies. At 24 h, the absorbed dose was 0.04% (0.72 ng equiv/cm^2) of the applied dose.[4]

Absorption, Distribution, Metabolism, and Excretion

Toxicokinetic data (oral exposure) are presented in Table 5.

Animal

Oral

In rats, the principal route of excretion of radioactivity from radiolabeled Polyaminopropyl Biguanide (as PHMB HCl) was in the feces. In one study, rats were dosed orally with 20 mg/kg/day for 10 days and elimination after dosing was described as follows: 5.6% ± 0.35% excreted in urine, 93.1%± 1.58% excreted via feces and 0.2 % exhaled. In another animal study (species not stated) of the distribution of radioactivity after dosing, the greatest amounts of radioactivity were detected in adipose tissue, followed by the kidneys and liver. No radioactivity was detected in brain. Small amounts of Polyaminopropyl Biguanide (as PHMB HCl) oligomers with 2 cyanoguanidino end groups, as well as the trace constituents, 3,3-dicyano-1,1-hexamethylenediguanidine and a compound considered to be 1- (6-aminohexyl)-3-cyanoguanidine, were found in the urine.[4,17]

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Acute toxicity data are presented in Table 6 (dermal studies), Table **7** (oral studies), and Table 8 (inhalation studies).

Dermal

There was no mortality or systemic toxicity in rats that received a single dermal dose of 5000 mg/kg aqueous Polyaminopropyl Biguanide (as PHMB HCl); but hemorrhage of dermal capillaries at the application site was observed. In an acute dermal toxicity study on 20% aqueous Polyaminopropyl Biguanide (as PHMB HCl) on rabbits, the LD_{50} was reported to be > 400 mg/kg.[4,17]

Oral

An LD_{50} of > 1000 mg/kg was reported for rats dosed orally with aqueous solutions (up to 25% aqueous) of Polyaminopropyl Biguanide (as PHMB HCl). A median lethal dose of 25.6 mg/kg was reported for rats dosed orally with a solution of 0.4% Polyaminopropyl Biguanide (as PHMB).[4,16,17,18,19]

Risk Assessment

The EPA conducted a screening-level acute dietary human health risk assessment for Polyaminopropyl Biguanide (as PHMB) food uses.[2] Risk estimates were provided for females 13 to 50 years old, the only population subgroup with an acute toxicity endpoint that was of concern. Risk estimates at the highest exposures were 9% of the acute Population Adjusted Dose (aPAD), which was below the Agency's level of concern. The aPAD is defined as the dose at which an individual could be exposed on any given day and no adverse health effects would be expected.

Inhalation

An LC_{50} was reported to be > 0.36 mg/l in acute inhalation toxicity studies in which rats were exposed to Polyaminopropyl Biguanide (as PHMB HCl) solutions (concentrations up to 0.5 mg/l in air). Dark/red lungs were observed at necropsy. A dose-related depression of respiratory rate was reported in a study in which mice were exposed to Polyaminopropyl Biguanide (as PHMB HCl) at concentrations up to 208 mg/m^3.[4]

Short-Term Toxicity Studies

Short-term dermal, oral, and inhalation toxicity studies are presented in Table 9.

Dermal

In the longest-duration study involving rats, there were no mortalities or signs of systemic toxicity in rats administered a 0.4% solution of Polyhexamethylene Biguanide over a 60-day period. Similar results were reported for rats after dermal applications of Polyaminopropyl Biguanide (as PHMB HCl) at doses up to 200 mg/kg daily over a 30-day period (21 applications total; no-observed-adverse-effect-level (NOAEL) = 200 mg/kg). In a 21-day dermal toxicity study involving rabbits, there was no evidence of toxic effects on the skin after 20% aqueous Polyaminopropyl Biguanide (as PHMB HCl) was applied.[4,17]

Oral

A lowest-observed-adverse-effect-level (LOAEL) of 0.1 mg/ml for Polyaminopropyl Biguanide (as PHMB HCl) was reported in 28-day oral toxicity studies involving rats.[4,17,18]

Inhalation

In 21-day and 28-day inhalation toxicity studies on Polyaminopropyl Biguanide (as PHMB HCl) involving rats, NOAECs equal to 0.025 mg/m^3 and 0.0239 mg/m^3, respectively, were reported.[4]

Subchronic Toxicity Studies

Subchronic oral toxicity data are presented in Table 10.

Oral

The following results were reported in 90-day oral toxicity studies on Polyaminopropyl Biguanide (as PHMB HCl) involving rats: no mortalities, but iron pigment/deposits observed in Kupffer cells (at 12500 ppm and 5000 ppm in diet) and a NOAEC of 1000 ppm. There were no treatment-related macroscopic post-mortem findings in mice in a 90-day drinking water study on 20% aqueous Polyaminopropyl Biguanide (as PHMB HCl), and a NOAEC of 1000 ppm was reported for this ingredient in a 90-day feeding study in which mice received concentrations up to 4000 ppm in the diet. A NOAEC of 5500 ppm was reported for Beagle dogs fed Polyaminopropyl Biguanide (as PHMB HCl)at concentrations up to 11000 ppm in the diet for 90 days.[4,17]

Chronic Toxicity Studies

Results from chronic dermal and oral toxicity studies are presented in Table 11.

Dermal

In an 80-week chronic toxicity study (dermal applications 5 days/week), a mortality rate of 75% was reported for the highest dose group (10% Polyaminopropyl Biguanide (as PHMB HCl), 30 mg dose). The exophthalmos observed throughout the study was more severe in this group, but the results of histological examination of the eyes and gross and microscopic examination of the thyroids were negative.[17]

Oral

In a 104-week oral toxicity study, a NOAEL of 2000 ppm (highest concentration fed in diet) was reported for Polyaminopropyl Biguanide (as PHMB HCl). This concentration corresponded to a daily dose of 36 mg/kg/day in male rats,

used to calculate a margin of safety (MOS) of 46, and, more recently, MOS values of 258 and 227. A no-observed-effect-concentration (NOEC, for histopathologic changes) of 200 ppm was reported in a 122-week oral toxicity study involving rats fed Polyaminopropyl Biguanide (as PHMB HCl) at concentrations up to 2000 ppm in the diet. In a study involving mice, feeding with Polyaminopropyl Biguanide (as PHMB HCl) (concentrations up to 1000 ppm in diet) for 97 weeks did not cause any macroscopic changes in tissues examined. A no-observed-adverse effect-concentration (NOAEC) of 1500 ppm for Polyaminopropyl Biguanide (as PHMB HCl) was reported in a 1-year feeding study involving dogs, though treatment-related histopathological findings in the liver and kidneys were reported at dietary concentrations of 3000 ppm and 4500 ppm. In a 26-week feeding study involving dogs, dietary concentrations of 1500 ppm and 4500 ppm Polyaminopropyl Biguanide (as PHMB HCl) produced dose-related hepatotoxicity and nephrosis.[4,17,19]

Risk Assessment

In an EPA human health risk assessment, residential-handler and post-application exposure scenarios relating to pesticide (including Polyaminopropyl Biguanide (as PHMB HCl)) application were assessed using surrogate exposure data, maximum application rates (on product label), and standard assumptions.[2] The agency determined that all margins of exposure (MOEs) from dermal and inhalation exposure for residential handlers are above the target MOE of 100, and, therefore, were not of concern. For post-application dermal and incidental ingestion (oral exposures) scenarios, MOE's were also below the Agency's level of concern. Residential handler exposures may occur when individuals mix, load, or apply a pesticide. Individuals could incur post-application exposure either as bystanders affected by application of the pesticide or when they enter a treated site.

Chronic dietary risk estimates were provided for the general United States population and all population subgroups.[2] It was determined that chronic dietary risk estimates are below the Agency's level of concern for the general U. S. population (<10% of the chronic Population Adjusted Dose [cPAD]) and all population subgroups (37% of the cPAD for children). The cPAD is the level of exposure (mg/kg/day) that should not be exceeded.[2]

The 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. ARIs were greater than 1.2 for children and 5.4 for adults, and these risks were determined to be below the Agency's level of concern (ARI < 1).[2]

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Developmental and reproductive toxicity data are presented in Table 12.

In oral reproductive and developmental toxicity studies on Polyaminopropyl Biguanide (as PHMB HCl) involving rats, NOAECs of 1,000 ppm and 1300 ppm have been reported. In an inhalation study, degeneration of seminiferous tubules in the testis of 1 male rat was observed at a concentration of 0.25 mg/m^3, but this toxic effect was not observed in any other group, including the highest concentration group (26 mg/m^3). NOAELs of 10 mg/kg/day and 40 mg/kg/day for developmental toxicity were reported in studies involving mice, and the higher dose was also classified as non-teratogenic in mice in another study. A NOAEL of 40 mg/kg/day for developmental toxicity has also been reported in a study involving rabbits. Polyhexamethylene Biguanide (as PHMB) has been classified as embryotoxic at oral dosage rates of 32 mg/kg/day (animal strain not stated) and 100 mg/kg/day (rats), and as teratogenic in rats at an intraperitoneal dosage rate of 10 mg/kg/day.[4,1,17,19]

GENOTOXICITY STUDIES

Genotoxicity data (in vitro and in vivo studies) are presented in Table 13.

In the Ames test, Polyhexamethylene Biguanide Hydrochloride (as PHMB HCl) was non-genotoxic 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, Polyhexamethylene Biguanide Hydrochloride (as PHMB HCl) was weakly genotoxic in strain 1538 without metabolic activation. Polyhexamethylene Biguanide (as PHMB) was non-genotoxic in the mouse lymphoma assay at concentrations up to 2000 µg/ml with and without metabolic activation, and in the in vitro micronucleus test at concentrations up to 50 µg/ml (without metabolic activation) and up to 250 µg/ml (with metabolic activation). In the in vivo micronucleus test, Polyhexamethylene Biguanide Hydrochloride (as PHMB HCl) was non-clastogenic in polychromatic erythrocytes from mice

that received single oral dosages up to 400 mg/kg. In the 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.[4]

CARCINOGENICITY STUDIES

Carcinogenicity data (in vitro, dermal and oral studies) are presented in Table 14.

In Vitro

Polyaminopropyl Biguanide (as PHMB HCl) was evaluated at concentrations up to 3000 µg/ml in the cell transformation assay (using baby hamster kidney fibroblasts), and there was no difference in the number of transformed cell colonies between test and negative control cultures. In another assay involving RAW 264.7 mouse macrophages, Polyaminopropyl Biguanide (as PHMB HCl), 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.[1,4]

Dermal

Polyhexamethylene Biguanide Hydrochloride (as PHMB HCl) was classified as a hepatic tumorigen in mice, i.e., at the highest dose (30 mg of 10% Polyhexamethylene Biguanide (as PHMB HCl) in ethanol) that was applied to the skin daily (5 days/week) for 80 weeks. An increase in the incidence of liver tumors was observed at the 30 mg/day dose; the increase was statistically significant only for liver tumors of endothelial origin. High mortality (76 to 78% of animals died) was noted in this group.[4,17]

Oral

A statistically significant increase in the incidence of hemangiosarcomas and hemangiomas was reported for only male mice that received Polyaminopropyl Biguanide (as PHMB HCl) at a dietary concentration of 4000 ppm daily for 2 years. In a 97-week study in which mice were fed Polyaminopropyl Biguanide (as PHMB HCl) at dietary concentrations up to 1000 ppm prior to and during mating, and their offspring were fed the same concentrations, there were no treatment-related (non-neoplastic or neoplastic) increases in histopathologic findings. Hemangiosarcomas or hemangiomas in the liver or other sites and a mortality incidence (80%) was reported by week 97. A concentration -related increase (100 to 1000 ppm) in tumor-bearing mice was reported in a similar 97-week dietary study. In a shorter-term feeding study (14 days), increased cell proliferation was noted at a concentration of 1200 ppm Polyaminopropyl Biguanide (as PHMB HCl) in the diet. Polyaminopropyl Biguanide (as PHMB HCl) was classified as non-carcinogenic in rats fed dietary concentrations up to 2000 ppm for 122 weeks. At 124 weeks, 80% mortality overall was reported. A low incidence of hemangiomas and hemangiosarcomas was reported in a study in which rats were fed Polyaminopropyl Biguanide (as PHMB HCl) at a dietary concentration of 2000 ppm for 2 years.[4,1,17,20]

OTHER RELEVANT STUDIES

Effect on Lung Cells

A study was performed to elucidate the inflammatory responses and its mechanisms induced by Polyaminopropyl Biguanide (as PHMB HCl) in lung cells.[21] A549 cells that were exposed to Polyaminopropyl Biguanide (as PHMB) showed concentration-dependent (0 to 80 µg/mL) decreased viability, significant reactive oxygen species (ROS) generation (at 20 µg/mL), inflammatory cytokine secretion (significant increase in 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]). Significant cytotoxicity to A549 cells was observed at concentrations >10 µg/mL. Polyaminopropyl Biguanide (as PHMB) triggered inflammatory cytokine secretion and NF-κB activation by modulating the degradation of IκB-α and the accumulation of nuclear p65. It was noted that TNF-α plays important roles in interleukin 8 (IL-8) expression as well as NF-κB activation. IL-8 production induced by Polyaminopropyl Biguanide (as PHMB) was completely suppressed by a NF-κB inhibitor, but not by a ROS scavenger. The authors suggested that Polyaminopropyl Biguanide (as PHMB) induces inflammatory responses via the NF-κB signaling pathway.

Cytotoxicity and Antimicrobial Activity

Polyaminopropyl Biguanide was compared to the (molecularly) closely related Polyaminopropyl Biguanide (as PHMB) with respect to antiseptic efficacy and cytotoxicity *in vitro*.[22] Antimicrobial efficacy tests were performed via determination of the minimum bactericidal concentration (MBC). Polyaminopropyl Biguanide (as PHMB) exhibited high antimicrobial activity against *S. aureus* and *E. coli*., whereas, though chemically closely related, Polyaminopropyl Biguanide proved to be ineffective in bacterial eradication. These results suggest that even small changes in the chemical structure of related agents, such as Polyaminopropyl Biguanide (as PHMB) and Polyaminopropyl Biguanide, can substantially affect their efficacy.

Cytotoxicity was evaluated in human keratinocytes (HaCaTs) and murine fibroblasts (L929). In fibroblast or ketatinocyte cultures, concentrations for both test substances ranged from 0.005% to 1% v/vand at concentrations ranging from 0.25% to 3% v/v. Cultures were incubated for up to 72 h. For all tested concentrations, Polyaminopropyl Biguanide (as PHMB) was highly cytotoxic to human HaCaT and L929 murine fibroblast cell after 24 and 72 h of incubation, never exceeding a survival rate of 27 %. Polyaminopropyl Biguanide displayed significantly lower cytotoxicity at concentrations ranging from 0.005% to 0.1% v/v. At concentrations up to 0.1 %, no cytotoxic effect could be detected in L929 cells after 24 h, whereas, for HaCaT cells, moderate and high cytotoxicity was evident at 0.05% and 0.1% Polyaminopropyl Biguanide. After 72 h, only a weak cytotoxic effect on L929 cell at 0.05% and 0.1% Polyaminopropyl Biguanide could be observed, while, for HaCaT cells, concentrations up to 0.1% were classified as non-cytotoxic. However, concentrations $\geq$ 0.25% Polyaminopropyl Biguanide were highly cytotoxic to cells of both cell lines after 24 h of incubation. When compared directly, Polyaminopropyl Biguanide consistently resulted in a significantly higher cell survival rate than Polyaminopropyl Biguanide (as PHMB), irrespective of concentration and incubation time ($P \leq 0.0006$).[22]

Epigenetic Effects

It has been proposed that Polyaminopropyl Biguanide (as PHMB HCl) be classified as a category 3 carcinogen though Polyaminopropyl Biguanide (as PHMB HCl) is not genotoxic.[23] It has been hypothesized that Polyaminopropyl Biguanide (as PHMB HCl) may have epigenetic effects, including non-genotoxic modifications of DNA bases, DNA methylation and mitogenic cytokine production. These properties have been assessed in vitro using 3 cell types: Caco-2 cells (from a human colon adenocarcinoma) with a non-functional p53 gene (Δp53: mut p53), N2-A (Neuro-2A cells, mouse neural cells), the brain being a possible target organ in rodents, and HepG2 cells (human hepatocellular carcinoma) with functional p53 gene. At Polyaminopropyl Biguanide (as PHMB HCl) concentrations of 1µg/mL to 20µg/mL, no effect was observed, neither growth stimulation nor inhibition. Viability testing using neutral red resulted in an IC_{50} of 20–25 µg/mL after treatment with Polyaminopropyl Biguanide (as PHMB HCl) for 3 h, 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. Polyaminopropyl Biguanide (as PHMB HCl) does not induce significant oxidative stress (as determined by measuring production of malondialdehyde (MDA) or lipoperoxidation, nor does it induce hydroxylation of DNA (8-hydroxy-2'-deoxyguanosine [8-OH-dG]) and/or its hypermethylation (5-methylcytosine [m5dC] content), the latter being strongly implicated in DNA replication and regulation and cell division.

Polyaminopropyl Biguanide (as PHMB HCl) 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, from concentrations of 20 to 100 µg/mL, Polyaminopropyl Biguanide (as PHMB HCl) killed cells of all types in less than 3 h. The expression of genes involved in the mechanisms of cell death induced by Polyaminopropyl Biguanide (as PHMB HCl), including p53, the pro apoptotic gene bax and others, the anti-apoptotic bcl-2 and caspase-3 genes has been evaluated using reverse transcription polymerase chain reaction (RT-PCR) methodology. Finally, the status of GAP-junctions (GJIC) in the presence of Polyaminopropyl Biguanide (as PHMB HCl) has been determined and appeared to not be significantly affected. Taken together, the data indicate that Polyaminopropyl Biguanide (as PHMB HCl) did not exhibit clear or remarkable epigenetic properties, except for a slight increase in the levels of some cytokines and a transcription factor at higher concentrations at which cell lysis occurs rapidly.[23]

DERMAL IRRITATION AND SENSITZATION STUDIES

Skin irritation, sensitization, and phototoxicity/photosensitization data are presented in Table 15.

Irritation

Polyaminopropyl Biguanide (as PHMB HCl) (single 4-h application) was classified as a mild skin irritant in rabbits. Single applications (24 h) of 20% aqueous Polyaminopropyl Biguanide (as PHMB HCl) to rabbits indicate that this compound is non-corrosive, moderately irritating to intact skin, and severely irritating to abraded skin. Repeated applications of Polyaminopropyl Biguanide (as PHMB HCl) (12,000 ppm) to the skin of rabbits for 21 days were not irritating. Severe

skin irritation was observed in all rats that received a single 24-h application of 25% aqueous Polyaminopropyl Biguanide (as PHMB HCl), at dosages of 2.5 ml/kg and 5 ml/kg. Polyaminopropyl Biguanide (as PHMB HCl) (0.04%) was classified as a non-irritant when applied to the skin of rats for 24 h. Repeated applications of 20.2% aqueous Polyaminopropyl Biguanide (as PHMB HCl) to rats for 21 days resulted in slight skin irritation (at 60 mg/kg/day) and moderate irritation (at 200 mg/kg/day). Slight to moderate erythema was observed in guinea pigs that received repeated applications of 25% aqueous Polyaminopropyl Biguanide (as PHMB HCl) for 3 days. In a study involving mice, the highest dose of Polyaminopropyl Biguanide (as PHMB HCl) (10% concentration in ethanol, 30 mg dose) caused hyperkeratosis and, occasionally, ulceration extending into the dermis when applied repeatedly for 80 weeks. Polyaminopropyl Biguanide (as PHMB HCl) (up to 1.5% active) was not classified as a primary skin irritant when applied for 24 h to the skin of human subjects.[1,4,17,24]

Sensitization

Polyaminopropyl Biguanide (as PHMB) was classified as a non-sensitizer in the local lymph node assay. In maximization tests, moderate skin sensitization was observed in guinea pigs challenged with Polyaminopropyl Biguanide (as PHMB HCl) (20.2 % active ingredient) and a 30% solution of the ingredient in deionized water, and moderate to strong sensitization was observed in guinea pigs challenged with Polyaminopropyl Biguanide (as PHMB) (20.2% active ingredient). In another guinea pig maximization test, Polyaminopropyl Biguanide (as PHMB HCl) (10% or 20%) was classified as a non-sensitizer. In Buehler sensitization tests, Polyaminopropyl Biguanide (as PHMB HCl) (2% active ingredient) was classified as a moderate sensitizer in guinea pigs, and the threshold for eliciting sensitization in guinea pigs was determined to be ~ 1%. Results from a study evaluating the possible cross-reactivity of Polyaminopropyl Biguanide (as PHMB HCl) (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 Polyaminopropyl Biguanide (as PHMB HCl) (2% active ingredient) was not capable of causing primary skin irritation, but was capable of causing sensitization. It was also determined that skin sensitization in humans can be elicited at concentrations beginning at 0.2% active ingredient.[4,25,26,27,28]

Photosensitization/Phototoxicity

Animal

Very strong irritation potential, but no significant photoirritancy, was reported in a study in which male rats were tested with Polyaminopropyl Biguanide (as PHMB HCl) at concentrations of 2% and 5%. When tested at a concentration of 1% in 26 subjects, Polyaminopropyl Biguanide (as PHMB HCl) was essentially non-irritating and did not induce sensitization, phototoxicity, or photoallergenicity.[17]

OCULAR IRRITATION STUDIES

Ocular irritation data are presented in Table 16.

Undiluted Polyaminopropyl Biguanide (as PHMB HCl) was a severe ocular irritant/corrosive agent when instilled into the rabbit eye. The instillation of 25% aqueous Polyaminopropyl Biguanide (as PHMB HCl) into the eyes of rabbits resulted in severe inflammation and corneal damage in unrinsed eyes and slight inflammation in rinsed eyes. Moderate and mild ocular irritation were observed in unrinsed and rinsed rabbit eyes, respectively, after 20% aqueous Polyaminopropyl Biguanide (as PHMB HCl) was instilled. In another study involving rabbits, the instillation of Polyaminopropyl Biguanide (as PHMB HCl) (20% aqueous) into the eyes induced slight inflammation, but no corneal ulceration. Ocular irritation was not observed when Polyaminopropyl Biguanide (as PHMB HCl) (0.04% active ingredient) was instilled into the eyes of rabbits. In a study in which 20% aqueous Polyaminopropyl Biguanide (as PHMB HCl) was instilled into human eyes and the eyes of rabbits in a temperature-controlled chamber (32-36˚C), normal corneal morphology was observed at histological examination.[4,17,29]

CLINICAL STUDIES

Patient multicenter studies are presented in the Human Sensitization Studies section section of Table 15.

Retrospective and Multicenter Studies

The results of patient multicenter studies (study populations ranging from 374 to 1975) have indicated a low incidence of skin sensitization reactions to Polyaminopropyl Biguanide (as PHMB HCl).[4,26,27,28]

Case Reports

A male patient had a history of angioedema and pruritus after using wet wipes.[13] Patch test results for an ingredient of the wipes, Polyaminopropyl Biguanide (as PHMB) (tested at 1:10 in water), and the wipe 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 h.

A chronic, recurrent and itchy dermatitis was observed in a male patient who used wet wipes.[30] Polyaminopropyl Biguanide (as PHMB), an ingredient of the product, was tested at different concentrations (20%, 2% and 0.2% aqueous). Readings were performed in accordance with International Contact Dermatitis Research Group guidelines. At day 2 and day 4, respectively, + and ++ reactions to 20% Polyaminopropyl Biguanide (as PHMB) (with a papulovesicular reaction, extending outside of the test chamber) were observed; +? and + reactions to 2% Polyaminopropyl Biguanide (as PHMB) were observed on days 2 and 4, respectively. No reactions to 0.2% Polyaminopropyl Biguanide (as PHMB) were observed.

Two cases of severe anaphylaxis were reported following contact of a surgical wound with a hospital disinfectant containing 0.2 % Polyaminopropyl Biguanide (as PHMB).[16,31]

A female patient experienced symptoms of a grade III anaphylaxis 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 PHMB) as a disinfectant.[16,32]

No adverse effects were noted following the exposure of 29 patients to a pre-operative antiseptic for cataract surgery that contained 0.2 % Polyaminopropyl Biguanide (as PHMB).[33]

Other Clinical Reports

Based on medical surveillance information, obtained between 2004 and 2007, from employees who came in contact with Polyhexamethylene Biguanide Hydrochloride (as PHMB HCl) in the workplace, no cases of skin sensitization to this chemical were reported.[4] All manufacturing and laboratory employees were offered complete medical evaluations on a regular basis depending on their age. These were conducted every one to two years.

In a clinical trial (106 dialysis patients) in which patients were treated for infections, Polyaminopropyl Biguanide (as PHMB) was well-tolerated and there were only two cases of transient local skin erythema.[34] Four of 28 patients were excluded from a cohort study due to adverse effects related to a Polyhexamethyene Biguanide dressing.[35]

Reportedly, the application of very high doses of Polyaminopropyl Biguanide (as PHMB) can trigger fever and a generalized exanthema.[19]

SUMMARY

The safety of Polyaminopropyl Biguanide, used as a preservative in cosmetic products, is reviewed in this safety assessment. Polyaminopropyl Biguanide is the International Nomenclature of Cosmetic Ingredients (INCI) name for an amino polymer comprising propyl biguanide repeat units (polyaminopropyl biguanide (PABA)) ***or*** hexyl biguanide repeat units (polyhexamethylene biguanide (PHMB)), ***or*** hydrochloride salts thereof. The overwhelming majority of information/data identified in the published literature for inclusion in this safety assessment are on Polyaminopropyl Biguanide (as PHMB HCl), and these data are being used to evaluate the safety of all chemicals identified by the ingredient name Polyaminopropyl Biguanide in cosmetic products.

Polyaminopropyl Biguanide, in its neat form, represents a solid/powder of > 94.2 % purity, and is usually marketed as an approximately 20% aqueous solution. One method for manufacturing Polyaminopropyl Biguanide (as PHMB HCl) 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 2016 VCRP data, Polyaminopropyl Biguanide is being used in 128 cosmetic products, mostly leave-on products. The results of a concentration of use survey provided in 2016 indicate that Polyaminopropyl Biguanide is being used at concentrations up to 0.5% in both rinse-off and leave-on products.

In 2016, the SCCS issued a revised opinion (preliminary opinion) stating that the use of Polyaminopropyl Biguanide as a preservative in all cosmetic products at concentrations up to 0.1% is safe. The opinion also states that, because no new safety data on inhalation are available on Polyaminopropyl Biguanide, its use in sprayable formulations is not advised. The SCCS report recites both Polyaminopropyl Biguanide and Polyaminopropyl Biguanide (as PHMB HCl).

The safety of Polyaminopropyl Biguanide (as PHMB HCl) has been reviewed by the United States Environmental Protection Agency (EPA), and the Agency concluded that this pesticide has very low aggregate risk of adverse health effects to the public or environment.

The skin penetration of Polyaminopropyl Biguanide (as PHMB HCl) in vitro has been demonstrated using rat and human skin. The principal route of excretion of radioactivity from orally administered Polyhexamethylene Biguanide Hydrochloride (radiolabeled) was in the feces in rat studies. The following components have been detected in the urine of rats fed Polyaminopropyl Biguanide (as PHMB HCl) in the diet: oligomers with 2 cyanoguanidino end groups, as well as the trace constituents, 3,3-dicyano-1,1-hexamethylenediguanidine and a compound that was considered to be 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 (as PHMB HCl); but, hemorrhage of dermal capillaries at the application site was observed. In an acute dermal toxicity study on 20% aqueous Polyaminopropyl Biguanide (as PHMB HCl) involving rabbits, an LD_{50} > 400 mg/kg was reported.

The LD_{50} was reported to be > 1000 mg/kg for rats dosed orally with aqueous solutions (up to 25% aqueous) of Polyaminopropyl Biguanide (as PHMB HCl). A median lethal dose of 25.6 mg/kg was reported for rats dosed orally with a solution of 0.4% Polyaminopropyl Biguanide (as PHMB).

An LC_{50} of > 0.36 mg/l was reported in acute inhalation toxicity studies in which rats were exposed to Polyaminopropyl Biguanide (as PHMB) solutions (concentrations up to 0.5 mg/l). Dark/red lungs were observed at necropsy. A dose-related depression of respiratory rate was reported in a study in which mice were exposed to Polyaminopropyl Biguanide (as PHMB HCl) at concentrations up to 208 mg/m^3.

In a study involving A549 lung cells in vitro, it was noted that Polyaminopropyl Biguanide (as PHMB) induces inflammatory responses via the NF-κB signaling pathway.

In the longest-duration study involving rats, there were no mortalities or signs of systemic toxicity in rats administered a 0.4% solution of Polyaminopropyl Biguanide (as PHMB) over a 60-day period. Similar results were reported for rats after dermal applications of Polyaminopropyl Biguanide (as PHMB HCl) at doses up to 200 mg/kg daily over a 30-day period (21 applications total; no-observed-adverse-effect-level (NOAEL) = 200 mg/kg). In a 21-day dermal toxicity study involving rabbits, there was no evidence of toxic effects on the skin after 20% aqueous Polyaminopropyl Biguanide (as PHMB HCl) was applied.

An LOAEL = 0.1 mg/ml for Polyaminopropyl Biguanide (as PHMB HCl) was reported in 28-day oral toxicity studies involving rats.

In 21-day and 28-day inhalation toxicity studies on Polyaminopropyl Biguanide (as PHMB HCl) involving rats, NOAEL values of 0.025 mg/m^3 and 0.0239 mg/m^3, respectively, were reported.

In 90-day toxicity studies on rats and mice, 4000 to 5000 ppm Polyaminopropyl Biguanide (as PHMB HCl) or more in the diet was associated with iron pigment deposits in Kupffer cells in the rats, but no mortalities; the NOAEL was 1000 ppm in both species. In a 60-day and a 90-day study, 0.4% and 20% Polyaminopropyl Biguanide (as PHMB HCl) in drinking water yielded no signs of toxicity in rats and mice, respectively. A NOAELof 5500 ppm was reported for Beagle dogs fed up to11000 ppm Polyaminopropyl Biguanide (as PHMB HCl) in the diet for 90 days.

In an 80-week chronic toxicity study (dermal applications 5 days/week), a mortality rate of 75% was reported for the highest dose group (10% Polyaminopropyl Biguanide (as PHMB HCl), 30 mg dose). The exophthalmos observed throughout the study was more severe in this group, but the results of histological examination of the eyes and gross and microscopic examination of the thyroids were negative.

In a 104-week oral toxicity study, a NOAEL of 2000 ppm (highest concentration fed in diet) was reported for Polyaminopropyl Biguanide (as PHMB HCl). This concentration corresponded to a daily dose of 36 mg/kg/day in male rats, used to calculate a margin of safety (MOS) of 46, and, more recently, MOS values of 258 and 227. A NOEL (for histopathologic changes) of 200 ppm was reported in a 122-week oral toxicity study involving rats fed Polyaminopropyl Biguanide (as PHMB HCl) at concentrations up to 2000 ppm in the diet. In a study involving mice, feeding with Polyaminopropyl Biguanide (as PHMB HCl) (concentrations up to 1000 ppm in diet) for 97 weeks did not cause any macroscopic changes in tissues examined. A NOAEL of 1500 ppm for Polyaminopropyl Biguanide (as PHMB HCl) was reported in a 1-year feeding study involving dogs, though treatment-related histopathological findings in the liver and kidneys were reported at dietary concentrations of 3000 ppm and 4500 ppm. In a 26-week feeding study involving dogs, dietary concentrations of 1500 ppm and 4500 ppm Polyaminopropyl Biguanide (as PHMB HCl) produced dose-related hepatotoxicity and nephrosis.

In oral reproductive and developmental toxicity studies on Polyaminopropyl Biguanide (as PHMB HCl) involving rats, NOAEL values of 1,000 ppm and 1300 ppm have been reported. In an inhalation study, degeneration of seminiferous tubules in the testis of 1 male rat was observed at a dose of 0.25 mg/m^3, but this toxic effect was not observed in any other dose group, including the highest dose group (26 mg/m^3). NOAEL values of 10 mg/kg/day and 40 mg/kg/day for developmental toxicity were reported in studies involving mice, and the higher dose was also classified as non-teratogenic in mice in another study. A NOAEL of 40 mg/kg/day for developmental toxicity has also been reported in a study involving rabbits. Polyaminopropyl Biguanide (as PHMB) has been classified as embryotoxic at oral doses of 32 mg/kg/day (animal strain not stated) and 100 mg/kg/day (rats), and as teratogenic in rats at an intraperitoneal dose of 10 mg/kg/day.

In the Ames test, Polyaminopropyl Biguanide (as PHMB HCl) was non-genotoxic 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 (as PHMB HCl) was weakly genotoxic in strain 1538 without metabolic activation. Polyaminopropyl Biguanide (as PHMB) was non-genotoxic in the mouse lymphoma assay at concentrations up to 2000 µg/ml with and without metabolic activation, and in the in vitro micronucleus test at concentrations up to 50 µg/ml (without metabolic activation) and up to 250 µg/ml (with metabolic activation). In the in vivo micronucleus test, Polyaminopropyl Biguanide (as PHMB HCl) was non-clastogenic in polychromatic erythrocytes from mice that received single oral doses up to 400 mg/kg. In the 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 (as PHMB HCl) was evaluated at concentrations up to 3000 µg/ml in the cell transformation assay (using baby hamster kidney fibroblasts), and there was no difference in the number of transformed cell colonies between test and negative control cultures. In another assay involving RAW 264.7 mouse macrophages, Polyaminopropyl Biguanide (as PHMB HCl), 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.

Except for a slight increase in some cytokines and transcription factor at concentrations at which cell lysis occurs rapidly, Polyaminopropyl Biguanide (as PHMB HCl) did not exhibit clear and remarkable epigenetic properties.

Polyaminopropyl Biguanide (as PHMB HCl) was classified as a hepatic tumorigen in mice, i.e., at the highest dose (30 mg of 10% Polyaminopropyl Biguanide (as PHMB HCl) in ethanol) that was applied to the skin daily (5 days/week) for 80 weeks. An increase in the incidence of liver tumors was observed at the 30 mg/day dose; the increase was statistically significant only for liver tumors of endothelial origin. High mortality (76 to 78% of animals died) was noted in this group.

A statistically significant increase in the incidence of hemangiosarcomas and hemangiomas was reported for only male mice that received Polyaminopropyl Biguanide (as PHMB HCl) at a dietary concentration of 4000 ppm daily for 2 years. In a 97-week study in which mice were fed Polyaminopropyl Biguanide (as PHMB HCl) at dietary concentrations up to 1000 ppm prior to and during mating and their offspring were fed the same concentrations, there were no treatment-related (non-neoplastic or neoplastic) increases in histopathologic findings. Hemangiosarcomas or hemangiomas in the liver or other sites were reported in this study along with the high mortality incidence (80%) by week 97. A concentration-related increase (100 to 1000 ppm) in tumor-bearing mice was reported in a similar 97-week dietary study. In a shorter-term feeding study (14 days), increased cell proliferation was noted at a concentration of 1200 ppm Polyaminopropyl Biguanide (as PHMB HCl) in the diet. Polyaminopropyl Biguanide (as PHMB HCl) was classified as non-carcinogenic in rats fed dietary concentrations up to 2000 ppm for 122 weeks. At 124 weeks, 80% mortality overall was reported. A low incidence of hemangiomas and hemangiosarcomas was reported in a study in which rats were fed Polyaminopropyl Biguanide (as PHMB) at a dietary concentration of 2000 ppm for 2 years.

A cytotoxicity assay was performed using human keratinocytes (HaCaTs) and murine fibroblasts (L929). When compared directly, Polyaminopropyl Biguanide (as PHMB) consistently resulted in a significantly higher cell survival rate (i.e., less cytotoxicity) than Polyaminopropyl Biguanide (as PHMB), irrespective of concentration and incubation time ($P \leq 0.0006$).

The results of animal studies indicate that the skin irritation potential of Polyaminopropyl Biguanide (as PHMB HCl) may be concentration-dependent as well as dependent upon the duration of application. For example, the skin irritation potential of Polyaminopropyl Biguanide (as PHMB HCl) (single 4-h application) was classified as a mild skin irritant in rabbits. Single applications (24 h) of 20% aqueous Polyaminopropyl Biguanide (as PHMB HCl) to rabbits were non-corrosive, moderately irritating to intact skin, and severely irritating to abraded skin. Repeated applications of Polyaminopropyl Biguanide (as PHMB HCl) (12,000 ppm) to the skin of rabbits for 21 days were classified as non-irritating. Polyaminopropyl Biguanide (as PHMB HCl) (up to 1.5% active) was not classified as a primary skin irritant when applied for 24 h to the skin of human subjects.

Polyaminopropyl Biguanide (as PHMB) was classified as a non-sensitizer in the local lymph node assay. However, results were mixed in animal studies. In maximization tests, moderate skin sensitization was observed in guinea pigs challenged with Polyaminopropyl Biguanide (as PHMB HCl) (20.2 % active ingredient) and a 30% solution of the ingredient in deionized water, and moderate to strong sensitization was observed in guinea pigs challenged with Polyaminopropyl Biguanide (as PHMB) (20.2% active ingredient). In another guinea pig maximization test, Polyaminopropyl Biguanide (as PHMB HCl) (10% or 20%) was classified as a non-sensitizer.

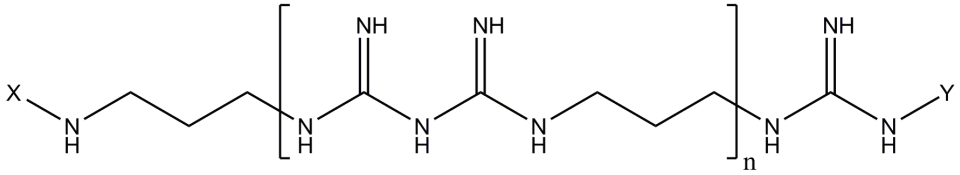
Very strong irritation potential, but no significant photoirritancy, was reported in a study in which male rats were tested with Polyaminopropyl Biguanide (as PHMB HCl) at concentrations of 2% and 5%. When tested at a concentration of 1%, in 26 subjects, Polyaminopropyl Biguanide (as PHMB HCl) was essentially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

Case reports with sensitization reactions to Polyaminopropyl Biguanide (as PHMB), an ingredient of wet wipes, and wet wipes containing this ingredient have been reported.

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

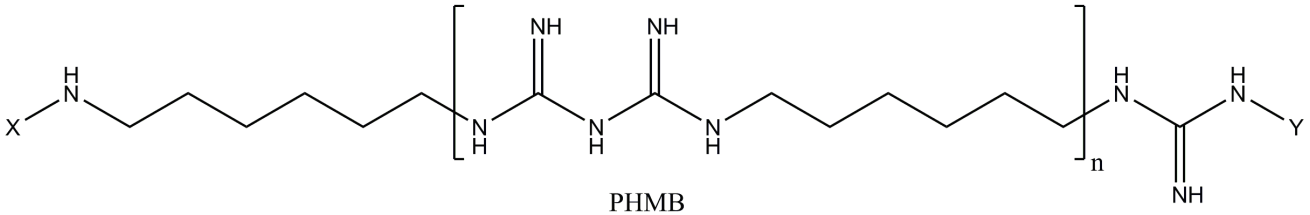
Table 1. Definition, idealized structures, and function of the ingredient in this safety assessment.[3; [CIR Staff]]

Ingredient CAS No.	Definition & Idealized Structures	Function
Polyaminopropyl Biguanide 133029-32-0 [PABA] 32289-58-0 [PHMB HCl] [27083-27-8 (PHMB HCl)] [28757-47-3 (PHMB)]	Polyaminopropyl Biguanide is the organic compound that conforms to [one of] the formula[s]. [Polyaminopropyl Biguanide is an amino polymer comprising propyl biguanide repeat units (polyaminopropyl biguanide (PABA)) or hexyl biguanide repeat units (polyhexamethylene biguanide (PHMB)), or hydrochloride salts thereof.]	Preservatives



PABA

or



PHMB

wherein X may be hydrogen or the hydrochloride salt, and Y may be nitrile or the hydrochloride salt.

Table 2. Physical and Chemical Properties of Polyaminopropyl Biguanide

Property	Value	Reference
physical form (at 20°C and 101.3 kilopascals [kPa])	liquid	[1]
color	pale yellow glass-like solid	[1]
average molecular weight (Daltons [Da])	3686-4216	[1]
water solubility (g/100 ml)	41 ± 1 %	[1]
other solubility (g/100 ml)	ethanol: 0.5 ± 0.08% methanol: 41 ± 1 %	[1]
relative density (at 20 ± 0.5°C)	1.20 ± 0.0025	[1]
melting point (°C)	78.9-136.3	[1]
boiling point (°C)	decomposes at 205-210°C before boiling	[1]
vapor pressure (Pa at 20°C)	1.32×10^{-7}	[1]
log P_{ow} (at 25 ± 1°C)	-2.3	[1]
UV absorption (λ) (nm)	236 nm	[1]

Table 3. Frequency and concentration of use according to duration and type of exposure

	# of Uses[6]	*Max Conc of Use (%)*[7]
	Polyaminopropyl Biguanide	
Totals*	**128**	**0.000002-0.5**
Duration of Use		
Leave-On	*87*	*0.000002-0.5*
Rinse-Off	*41*	*0.00025-0.5*
Diluted for (Bath) Use	*NR*	*NR*
Exposure Type		
Eye Area	25	0.01-0.3
Incidental Ingestion	1	NR
Incidental Inhalation-Spray	1	0.000002-0.27; 0.000023-0.5%*
Incidental Inhalation-Powder	NR	NR
Dermal Contact	97	0.00001-0.5
Deodorant (underarm)	NR	0.003
Hair - Non-Coloring	16	0.000002-0.5
Hair-Coloring	NR	0.5
Nail	2	NR
Mucous Membrane	1	NR
Baby Products	NR	0.1

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.

*It is possible these products are sprays, but it is not specified whether the reported uses are sprays

NR – not reported

Table 4. Dermal Penetration Studies

Ingredient	Animals/Protocol	Results
[^{14}C]-Polyhexamethylene Biguanide Hydrochloride 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 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]-Polyhexamethylene Biguanide Hydrochloride, absorption rates (ng/cm^2/h) through rat whole skin were 131, 3695, and 11940, respectively.[4]
[^{14}C]-Polyhexamethylene Biguanide Hydrochloride (5% solution)	Applied to skin biopsies of newborn, hairless rats and to human epidermal skin in diffusion chamber.	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 h, and this penetration rate was from 0.11 % up to 0.81 % after adding dimethylsulfoxide (DMSO).[4]
[^{14}C]-Polyhexamethylene Biguanide Hydrochloride (0.1% w/w in aqueous micellar solution)	0.1% in aqueous solution (24-h study) applied to human split-thickness skin from 4 donors (dose volume = 200 µl/cm^2; application rate ≈ 2 mg/cm^2) in diffusion cell.	48.3% and 52.35% of radioactivity, respectively, removed during washing procedure. At 24 h, absorbed dose was 0.03% (0.58 ng equiv/cm^2) and 0.04% (0.72 ng equiv/cm^2) of applied dose, respectively. Epidermis + lower layers of stratum corneum contained 11.47% (238 ng equiv/cm^2) and 14.20% (291 ng equiv/cm^2) of applied dose, respectively. Dermis contained 1.56% (32.3 ng equiv/cm^2) and 1.02% (20.9 ng equiv/cm^2) of applied dose, respectively. The mass balance was complete (90.93% and 98.96% of applied dose, respectively).[1]
[^{14}C]-Polyhexamethylene Biguanide Hydrochloride (0.1% w/w in oil-in-water emulsion)	0.3% in emulsion (72-h study) applied to human split-thickness skin from 4 donors (dose volume = 200 µl/cm^2; application rate ≈ 2 mg/cm^2) in diffusion cell.	53.33% and 58.10% of radioactivity, respectively, removed during washing procedure. At 72 h, absorbed dose was 0.02% (1.29 ng equiv/cm^2) and 0.03% (1.94 ng equiv/cm^2) of applied dose, respectively. Epidermis + lower layers of stratum corneum contained 14.54% (972 ng equiv/cm^2) and 14.45% (921 ng equiv/cm^2) of the applied dose, respectively. Dermis contained 1.23% (82 ng equiv/cm^2) and 1.46% (93.4 ng equiv/cm^2) of applied dose, respectively. The mass balance was complete (92.71% and 99.25% of applied dose, respectively).[1]
[^{14}C]-Polyhexamethylene Biguanide Hydrochloride (19.2% aqueous; specific activity = 38.9 mCi/g)	Applied to abdominal skin (from 7 human donors) as oil/water emulsion with labeled and non-labeled test substance (dose = 2.06 mg/cm^2). Split-thickness skin samples (380 to 400 µm) in static diffusion cells used.	At 24 h, the absorbed dose (mean: 0.17 %) was the sum of the receptor fluid (0.171 %) and the receptor wash (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 %).[4]

Table 4. Dermal Penetration Studies

Ingredient	Animals/Protocol	Results
[^{14}C]-Polyhexamethylene) Biguanide Hydrochloride (20.2% aqueous; specific activity = 1.85 GBq/732 mg)	Applied to human skin epidermal membranes in diffusion cell. 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 201 g active ingredient/l (occluded), absorption rate 0.110 ± 0.044 µg/cm^2/h (n = 4) and absorption percentage 0.001% over 24-h. 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-h .[4]
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride (20.2% aqueous; specific activity = 1.4 MBq/mg)	Test substance warmed to 40°C and applied (200 µl/cm^2, unocluded and occluded) to human skin epidermal membranes in diffusion cell.	At a of 200 g active ingredient/l (occluded for 0.5 h 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-h 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-h period. Recovery was not reported and was not possible to derive a realistic dermal absorption rate from this study.[4]

Table 5. Toxicokinetic Studies

Ingredient	Animals/Protocol	Results
[^{14}C]-Polyhexamethylene Biguanide Hydrochloride (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), single oral dose of low, medium or high MW fraction: 94.9%, 101.4%, and 96% of radioactivity from low, mid, and high MW fractions, respectively, eliminated via feces. 5.2%, 0.2%, and 0.2 % 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 h (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 dose.[4]
[^{14}C]-Polyhexamethylene Biguanide Hydrochloride (20% aqueous in double deionized water; specific activity = 1.85 GBq/4 mmol)	Groups of Alpk:APfSD (Wistar-derived) rats (5/sex/group) fed with 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 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 bw).	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 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.[4]
Radiolabeled Polyhexamethylene Biguanide Hydrochloride	5 male Alderley Park rats. Oral dosage rate 20 mg/kg/day over 10 days.	5.6% ± 0.35 % excreted in urine, 93.1% ± 1.58% excreted via feces and 0.2 % exhaled.[4]
Radiolabeled Polyhexamethylene Biguanide Hydrochloride	Animal species not stated. Feeding with diet containing 20 ppm.	Highest 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 Polyhexamethylene Biguanide Hydrochloride 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.[4]

Table 5. Toxicokinetic Studies

Ingredient	Animals/Protocol	Results
20% Polyhexamethylene Biguanide Hydrochloride (4.6 µCi)	5 male rats (strain not stated). Feeding with dosages of 100 mg/kg in the diet	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 enterhepatic recirculation.[17]
20% Polyhexamethylene Biguanide Hydrochloride	3 male rats (strain not stated) maintained on diet that contained 100 ppm test substance	Concentration in abdominal fat peak ed 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.[17]
[^{14}C]-Polyhexamethylene Biguanide Hydrochloride	10 NMRI mice received single oral dose of 2.0 mL by gavage. Whole body autoradiography	No absorption detected[4]

Table 6. Acute Dermal Toxicity Studies

Ingredient	Animals	Protocol	Results
Polyhexamethylene Biguanide Hydrochloride (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 body weight. Application site covered with semi-occlusive dressing for 24 h. 14-day observation period.	No mortalities or systemic toxicity. Hemorrhage of dermal capillaries noted at treatment sites of 8 animals one and two days after dosing.[4]
Polyhexamethylene Biguanide Hydrochloride (20% aqueous)	2 groups of 20 (10 males, 10 females) albino rats of SPF strain.	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 $inch^2$. Site covered with patch for 24 h. 7-day observation period. Necropsy not performed.	No mortalities.[17]
Polyhexamethylene Biguanide Hydrochloride (20% aqueous)	4 New Zealand White rabbits (2 males, 2 females).	OECD Guideline 402. Test substance (2 ml) applied to shaved area (~150 x 130 mm) of dorso-lumbar region and held in place with occlusive dressing for 24 h. 14-day observation period.	Dermal LD_{50} > 2 ml/kg body weight, i.e., greater than 400 mg/kg body weight (active ingredient).[4]

Table 7. Acute Oral Toxicity Studies

Ingredient	Animals	Protocol	Results
		Rats	
Polyhexamethylene Biguanide hydrochloride (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 body weight).	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 non-glandular 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.[4]
25% aqueous Polyhexamethylene Biguanide Hydrochloride	6 rats (3 males, 3 females; strain not stated)	Single oral dose of 4000 mg/kg body weight (equivalent to 1000 mg/kg Polyhexamethylene Biguanide Hydrochloride) 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 body weight Polyhexamethylene Biguanide Hydrochloride.[17]
25% aqueous Polyhexamethylene Biguanide Hydrochloride	3 female rats (strain not stated)	Single oral dose, followed by 7-day observation period.	No deaths and all organs appeared normal at necropsy.[17]
25% aqueous Polyhexamethylene Biguanide hydrochloride	6 rats (3 males, 3 females; strain not specified)	Single oral dose of 40000 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.[17]
20% aqueous Polyhexamethylene Biguanide hydrochloride	groups of Alderley Park rats (5 /sex/dose)	OECD Guideline 401. Doses up to 5000 mg/kg body weight (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} values of 2747 mg/kg (males) and 2504 mg/kg (females), corresponding to ~ 549 and ~501 mg/kg body weight (active ingredient), respectively.[4]
Polyhexamethylene Biguanide (in deionized water)	Groups of 10 Sprague-Dawley rats	Single dose by gavage (stomach tube). Doses ranged from 2 mg/kg to 40 mg/kg.	Administration 25.6 mg/kg dose, i.e. 1.6 mL of 0.4% Polyhexamethylene Biguanide solution (equivalent to 6.4×10^3 mg/l of 0.1% solution) resulted in 50% mortality. Median lethal dose (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.[18]

Table 8. Acute Inhalation Toxicity

Ingredient	Animals/Protocol	Results
Polyhexamethylene Biguanide Hydrochloride (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 for 4 h. Mass medium aerodynamic diameters: 1.49-2.20 µm, with GSD in 1.84-2.29 µm range.	Note: In preliminary test, 2 rats exposed to 1 mg/l died. At 0.1 mg/l, no deaths, but main clinical signs observed on day 0 and included: slight to moderately labored respiration, rhonchus, decreased activity, hunched back, and increased respiratory rate. At 0.3 mg/l, all animals with slight-to-moderately labored respiration. Slight-to -severe decreased activity also observed; moderate ataxia in one animal. At 0.5 mg/l, main clinical signs included: moderately -to-severely labored respiration with noisy respiration up to gasping, increased respiratory rate, and decreased activity. At necropsy, enlargement of dark/red discolored lungs and/or dark/red discoloration of the fur at the perinasal and/or white foamy material in the trachea in all animals found dead (only in 0.3 and 0.5 mg/l groups). LC_{50} = 0.37 mg/l for males and females combined.[4]
20.6% w/w Polyhexamethylene Biguanide Hydrochloride	Alpk:APfSC rats (10 rats; 5/sex). Exposed (nose-only) for 4 h to single dose of 1.76 mg/l of formulation defined as 0.36 mg/l of Polyhexamethylene Biguanide Hydrochloride (mass medium aerodynamic diameters: 1.8-2.0 µm, with a geometric standard deviation [GSD] of 2 µm)	1 male died 3 h after exposure. Respiratory distress in all females and most males. Red mottled lungs in dead male and 2 other males on day 15. Not possible to establish LC_{50} based on this study, but LC_{50} estimated at > 0.36 mg/l for Polyhexamethylene Biguanide Hydrochloride.[4]
Polyhexamethylene) Biguanide Hydrochloride (20% aqueous in spa water)	Groups of 5 mice of the Alpk:APfCD-1 strain exposed to aerosol. target concentrations 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} value (concentration causing 50 % depression in respiratory rate) 264 mg/m^3 (no sensory irritation) calculated.[4] The SCCS noted that this RD_{50} is outside of investigated concentration range and is of questionable reliability. SSCS also stated that the results of this study indicate that test substance should be considered a respiratory irritant.[4]

Table 9. Short-Term Toxicity Studies

Ingredient	Animals	Protocol	Results
Dermal Studies			
25% aqueous Polyhexamethylene Biguanide Hydrochloride	3 female rats (strain not stated).	Test substance applied (dose per cm^2 not stated) to intact skin of the back, under occlusive dressing, for 3 alternating 24-h periods; i.e., each application period followed by 24-h non-treatment period.	No specific systemic effects were observed.[17]
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 10 (5 males, 5 females per group) rats of the Alpk:APfSD (Wistar-derived) strain	Three groups received applications (occlusive, on back) at doses of 0 mg/kg, 20 mg/kg, 60 mg/kg, and 200 mg/kg, respectively, 6 h 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/ day.[4]
20% Polyhexamethylene Biguanide Hydrochloride (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 h.	No evidence of systemic toxicity (with or without covering).[17]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	female albino rabbits	12,000 ppm solution (1 ml) applied (unoccluded) to the back for 23 h. Re-applied, beginning at 1 h later, for total of 21 daily applications.	No evidence of toxic effects on the skin.[4]
Oral Studies			
25% aqueous Polyhexamethylene Biguanide Hydrochloride	14 rats (7 males, 7 females; strain no stated)	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, non-specific changes consistent with gastrointestinal inflammation.[17]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 16 (8 males, 8 females per group) rats of the Alpk:APfSD strain.	Four groups received doses of 0.1 mg/ml, 0.5 mg/ml, 1 mg/ml, 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 predominantly occurring 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.[4]

Table 9. Short-Term Toxicity Studies

Ingredient	Animals	Protocol	Results
20% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 20 (10 males, 10 females per group) mice of the C57Bl/10JfAP/alpk strain	Four groups received doses of 0.1 mg/ml, 0.3 mg/ml, 0.6 mg/ml, 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 effects). 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.[4]
Polyhexamethylene Biguanide (in deionized water)	Groups of 6 Sprague-Dawley rats	60-day study. Dosage rates: Group 1: 2 mg/kg (equivalent to 0.2 mg/l of 0.4% solution of test substance; Group 2: 8 mg/kg (equivalent to 0.4 mg/l of 0.4% solution of test substance; and Group 3: 32 mg/kg (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 with or without lymphocyte infiltration and feathery degeneration. Based on biochemical parameters examined, few deleterious effects in internal organs.[18]
Inhalation Studies			
19.2% aqueous Polyhexamethylene Biguanide Hydrochloride	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 h 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 concentrations of aqueous solutions corresponded to Polyhexamethylene Biguanide Hydrochloride concentrations of 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 post-mortem 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.[4]

Table 9. Short-Term Toxicity Studies

Ingredient	Animals	Protocol	Results
20% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 8 (4 males, 4 females per group) rats of the Alderley Park SPF albino strain.	Five groups exposed (nose-only) to doses of 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 h 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: slight-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. NOAEC = 0.025 mg/m^3.[4]

Table 10. Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
		Oral Studies	
25% Polyhexamethylene Biguanide Hydrochloride	Young adult specific pathogen free (SPF) Wistar rats (25 males, 25 females)	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.[17]
25% aqueous Polyhexamethylene Biguanide Hydrochloride	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.[4]
25% aqueous Polyhexamethylene Biguanide Hydrochloride	Three groups of Beagle dogs (4 males, 4 females per group)	90-day dietary study. Concentrations of 0 ppm, 5500 ppm, and 11000 ppm	No 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.[17]
25% aqueous Polyhexamethylene Biguanide Hydrochloride	Beagle dogs (inbred strain from Alderley Park, Cheshire). Groups of 4 dogs/sex/dose	90-day dietary study. Concentrations of 0, 1375 or 2750 ppm active ingredient as dietary admixture	No mortalities or signs of clear systemic toxicity.[4]
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	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 body weight/day active ingredient in males and 92.3, 192.9, 409.8, 617.4 mg/kg body weight/day 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 doses of 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 body weight/day in female rats).[4]

Table 10. Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	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 body weight/day active ingredient in males and 0, 224, 445, 963 mg/kg body weight/day active ingredient in females) and 6000 ppm active ingredient	Marked toxicity 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/day in male mice and 224 mg/kg/day in female mice) as NOAEL.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	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 post-mortem findings.[4]

Table 11. Chronic Toxicity Studies

Ingredient	Animals	Protocol	Results
		Dermal Study	
Polyhexamethylene Biguanide Hydrochloride	Four groups of specific pathogen free (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/day 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.[17]
		Oral Studies	
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	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 body weight/day (males) and 0, ~14.9, ~45.3, and ~162.3 mg/kg body weight/day (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 = 2000 ppm., corresponding to 36 and 45 mg/kg/day for males and females, respectively. The NOAEL of 36 mg/kg/day and systemic exposure dose (SED) of 0.0666 mg/kg/day used in calculation of margin of safety (MOS) for Polyhexamethylene Biguanide; MOS = 46.[4] In more recent MOS calculations, an SED of 0.012 mg/kg/day was used because the residual stratum corneum + epidermis fractions in more recent dermal penetration data were not considered as contributing to the SED. The new MOS values are 258 and 227 (i.e., lower when additional exposure from non-cosmetic use is incorporated).[1]

Table 11. Chronic Toxicity Studies

Ingredient	Animals	Protocol	Results
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	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 ppm, ~11 ppm, ~54 ppm, and ~169 or ~108 mg/kg/day) 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 animals that received 4500/3000 ppm. No treatment-related histopathological changes in dogs of 300 or 1500 ppm group. NOAEL = 1500 ppm.[4]
20% Polyhexamethylene Biguanide Hydrochloride	Groups of 30 male and 60 female Alderley Park mice of the SPF 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, i.e., 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 non-neoplastic findings (specific findings not stated) also not test substance-related.[17]
20% Polyhexamethylene Biguanide Hydrochloride	Four groups of Alderley Park rats of the SPF 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, i.e., 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, microsocpic 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.[17]

Table 11. Chronic Toxicity Studies

Ingredient	Animals	Protocol	Results
20% Polyhexamethylene Biguanide Hydrochloride	Four groups of adult Beagle dogs (4 males, 4 females per group)	26-week study. Dietary concentrations of 0 ppm, 500 ppm, 1500 ppm, 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 dose-related hepatotoxicity and nephrosis.[17]
Polyhexamethylene Biguanide	Strain not stated	Chronic toxicity study (protocol not stated).	NOEL = 200 mg/kg/day.[19]
Polyhexamethylene Biguanide	Strain not stated	2-year chronic toxicity study (protocol not stated). Dose: 100 mg/kg/day	No adverse effects.[19]

Table 12. Developmental and Reproductive Toxicity Studies

Ingredient	Animals/Embryos	Protocol	Results
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 52 (26 males, 26 females) rats 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/day in [males], and to 0, ~25-26, ~77-79, ~258-270 mg/kg/day [females] through 2 successive generations (including a 10-week pre-mating 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.[4]
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 20 female New Zealand White rabbits	Four groups received oral dosages (by gavage) of 0, 10, 20, and 40 mg/kg daily on gestation days 8 through 20.	No effect on the number of fetuses, growth or survival in utero, except a slight increase in pre-implantation loss observed at 40 mg/kg/day (21.8 ± 25.6 vs 13.1 ± 15.2 in controls) and a significant increase in postimplantation loss at 20 mg/kg (11.4 ± 19.7 % vs 6.1 ± 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/day, but results not considered test substance-related. Maternal NOAEL = 20 mg/kg/day. Developmental NOAEL = 40 mg/kg/day.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	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 pre-mating period and until the 3rd 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.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 20 rats of the Alderly 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 /day) 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.[4]

Table 12. Developmental and Reproductive Toxicity Studies

Ingredient	Animals/Embryos	Protocol	Results
20% aqueous Polyhexamethylene Biguanide Hydrochloride (in 0.5% aqueous polyoxyethylene(20)sorbitan monooleate)	Groups of 47 to 49 mice of the Alderly Park strain. Group of 25 mice served as the control.	Four groups received doses (by gavage) of 10, 20, and 40 mg/kg/day (expressed as active ingredient) on gestation days 6 through 15 (mating day considered gestation day 0).	No mortalities or test substance-related adverse clinical signs. Gestational parameters such as implantation sites, pre- and post implantation loss, litter size and weight, resorptions not influenced by test substance at any dose. Indications of slight retardation of ossification from examination of forelimb and hindlimb digits and numbers of caudal vertebrae at doses of 20 and 40 mg/kg /day. Maternal NOAEL = 40 mg/kg /day. Developmental NOAEL = 10 mg/kg/day.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride (in 0.5% aqueous polyoxyethylene(20)sorbitan monooleate)	Groups of 47 to 49 mice of the Alderly Park strain. Group of 25 mice served as the control.	Four groups received doses (route not stated) of 10, 20, and 40 mg/kg/day (expressed as active ingredient) on gestation days 6 through 15 (mating day considered gestation day 0).	Increased mortality (6 dams). No effect on number or growth or survival in utero, except slight increase, not statistically significant, in pre-implantation loss observed at 40 mg/kg (21.8 ± 25.6 vs. 13.1 ±15.2 in controls) and significant increase in postimplantation loss at 20 mg/kg (11.4 ± 19.7% vs. 6.1 ± 8.4% in controls), attributed to increase in early intrauterine deaths. Percentage of fetuses with unossified 5th sternebrae or with fused 4th and 5th sternebrae increased at 40 mg/kg, but not considered test substance-related. Maternal NOAEL = 20 mg/kg/day. Developmental NOAEL = 40 mg/kg/day.[1]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	Four groups of at least 21 pregnant mice of the Alderly strain	The following dosages were administered daily by gavage on gestation days 6 to 15: 0 (control), 10, 20, or 40 mg/kg.	Litter and fetal parameters similar in all groups. Soft tissue anomalies unremarkable. Skeletal examinations revealed anomalies of the skull, sternebrae, and hindlimb. Incidences in the 3 dose groups were double those noted in control group. Based on these results, retardation of ossification observed in each dose group considered by the authors to be marginal. No-effect-level for delayed ossification was not established. Test substance was classified as non-teratogenic.[4]

Table 12. Developmental and Reproductive Toxicity Studies

Ingredient	Animals/Embryos	Protocol	Results
20% Polyhexamethylene Biguanide Hydrochloride	Four groups of Sprague-Dawley albino rats (10 males and 20 females/group).	Three-generation reproduction study. 4 groups received test substance at dietary concentrations of 0, 200, 650, and 1300 ppm for 9 weeks, through 3 successive generations.	No effects attributable to test substance administration observed in relation to parental food consumption values, survival rates, clinical findings, pregnancy rates, or reproduction data. No meaningful differences between treated and control groups with respect to various reproductive indices, sex ratios, and body weight data for the fetuses. Necropsy of weanlings did not reveal test substance-related gross pathology. No findings indicative of embryotoxicity or teratogenicity. NOEL = 1300 ppm.[17]
0.04% Polyhexamethylene Biguanide	Animal strain not stated.	Oral dosing (test protocol not included)	Embryotoxic at dose of 32 mg/kg/day.[19]
Polyhexamethylene Biguanide	Rats (number and strain not stated)	Rats dosed orally with 100 mg/kg/day	Embryotoxic.[19]
Polyhexamethylene Biguanide	Rats (number and strain not stated)	Rats dosed intraperitoneally with 10 mg/kg/day	Teratogenic.[19]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 8 (4 males, 4 females per group) rats of the Alderley Park SPF albino strain.	In short-term toxicity study, 5 groups exposed (nose-only) to doses of 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 h 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.[4]

Table 13. Genotoxicity Studies

Ingredient/Similar Chemical	Strain/cell type	Assay	Dose/Concentration	Results
		In Vitro		
20% aqueous Polyhexamethylene Biguanide Hydrochloride	*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 dose of 333.3 mg per plate, particularly in strains TA98, TA100, and TA1535. Weakly genotoxic in strain TA1538 without metabolic activation.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	*Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538	Ames test, with and without metabolic activation	Doses up to 500µg/plate	Non-genotoxic.[4]
19.6% aqueous Polyhexamethylene Biguanide Hydrochloride (in DMSO)	*Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538.	Ames test, with and without metabolic activation	Doses up to 5000 µg/plate	Non-genotoxic, with or without metabolic activation in all but one strain. In strain TA98, negative results without metabolic activation, but slight responses (2.1 x background) observed with metabolic activation. Non-genotoxic.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	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. Non-genotoxic.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	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. Non-genotoxic.[4]
19.6% aqueous Polyhexamethylene Biguanide Hydrochloride	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. Non-genotoxic.[4]
		In Vivo		
19.6% aqueous Polyhexamethylene Biguanide Hydrochloride	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 body weight (dosage volume = 10 ml/kg body weight).	Non-clastogenic.[4]

Table 13. Genotoxicity Studies

Ingredient/Similar Chemical	Strain/cell type	Assay	Dose/Concentration	Results
19.6% aqueous Polyhexamethylene Biguanide Hydrochloride	Alpk:APfSD (Wistar-derived) rat hepatocyte cultures exposed to [^{3}H]-thymidine	Unscheduled DNA synthesis assay	Rats of the Alpk:APfSD (Wistar-derived) strain. Test substance administered (single dose, by gavage) to 2 - 3 males per dose at 0, 750, and 1500 mg/kg body weight (dosage volume = 10 ml/kg body weight) for 4 h or 12 h.	No induction of unscheduled DNA synthesis.[4]

Table 14. Carcinogenicity Studies

Ingredient	Animals/Cells	Protocol	Results
		In Vitro Studies	
20% aqueous Polyhexamethylene Biguanide Hydrochloride (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.[4]
Polyhexamethylene Biguanide Hydrochloride (up to 1 ppm)	RAW 264.7 mouse macrophages co-cultured with SVEC4-10 mouse endothelial cells.	Experiment 1: Preactivaton of macrophages with Polyhexamethylene Biguanide Hydrochloride (0, 0.75, and 1 ppm) or lipopolysaccharide (LPS) and/or co-culture in the presence of Polyhexamethylene Biguanide Hydrochloride. Endothelial proliferation analyzed by incorporation of bromodeoxyuridine (BrdU). Experiment 2 summarized below.	Polyhexamethylene Biguanide Hydrochloride had no direct effect on liver endothelial cell proliferation and did not potentiate cell proliferation induced by LPS-activated macrophages.[1]
Polyhexamethylene Biguanide Hydrochloride (up to 1 ppm)	RAW 264.7 mouse macrophages	Reactive oxygen species (ROS) assay. Macrophages cultured with Polyhexamethylene Biguanide Hydrochloride (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 (ELISA).	No activation of macrophages.[1]
		Dermal Studies	
Polyhexamethylene Biguanide Hydrochloride (up to 20%)	Four groups of specific pathogen free (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 (in ethanol), 0.6 mg (0.2% polyhexamethylene biguanide hydrochloride in ethanol), 6.0 mg (20% Polyhexamethylene Biguanide Hydrochloride) and 30 mg (10% Polyhexamethylene Biguanide Hydrochloride in ethanol).	Incidence of clinically-observed skin tumors: control (1 male), 6 mg of 20% concentration (2 males), and 30 mg of 10% concentration (1 male and 2 females). Liver + kidney tumors contributed more than 50% of total for the 30 mg dose group. Total number of kidney + liver tumors: control (5 males, 2 females), 0.6 mg group (4 males, 4 females), 6 mg 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 group; statistically significant (chi square, 1% level) only in case of liver tumors of endothelial origin (both benign and malig-nant; 2 in controls and 6 in 30 mg dose group). Many growths observed microscopically classified as moderate to severe hepatitis at histopathologic examination. Liver necrosis in all dose groups. Test substance classified

Table 14. Carcinogenicity Studies

Ingredient	Animals/Cells	Protocol	Results
			as hepatic oncogene in mice dosed with 30 mg.[17]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 100 (50 males, 50 females) mice of the Alpk:APfCD-1strain.	Four groups received dermal (non-occluded) dose rates of 0, 0.6, 6.0, and 30 mg/mouse/day (corresponding to 0, ~15, ~150, and ~750 mg/kg body weight/day) 5 days per week for 80 weeks.	High mortality (76-78 % of animals died) in 30 mg dose group. Variety of inflammatory hepatic changes in all groups, including controls. Severe hepatitis in some animals (number not stated) of 30 mg dose group. Slight increase in incidence of liver tumors observed at 30 mg/mouse/day (4 in the control; 10 in 30 mg dose group); statistically significant only in case of liver tumors of endothelial origin (both benign and malignant; 2 in control and 6 in 30 mg dose group). NOAEL = 0.6 mg/mouse/day.[4]
Oral Studies			
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 110 (55 males, 55 females) mice 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/day, respectively) daily for 2 years	Mortalities increased in 3000 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 (i.e., 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 these tumors in 1200 ppm group (considered a chance event). Some evidence of carcinogenicity.[4]
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 30 male and 60 female Swiss-derived albino mice	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	Study terminated when overall mortality reached 80 % at 97 weeks. High mortality due to fighting of males. No treatment-related (non-neoplastic 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. Data considered to be of low reliability due to high mortality.[4]

Table 14. Carcinogenicity Studies

Ingredient	Animals/Cells	Protocol	Results
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 30 male and 60 female Alderley Park mice of the SPF strain	Four 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; offspring fed same diet as parents throughout study	Study terminated at 97 weeks, when overall mortality reached 80%. 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]). 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.[17]
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 60 male and 60 female rats of unspecified strain	4 groups fed at concentrations of 0, 200, 1000 and 2000 ppm	Study terminated at 124 weeks, due to 80% mortality. 2 outbreaks of infection noted. Long-term exposure unrelated to toxic or carcinogenic 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.[4]
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	Wistar rats (20 males, 20 females)	Daily oral doses of 100 mg/kg 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 fact that only 20 rats per sex tested, no controls, and only 1 dose tested.[17]
20% Polyhexamethylene Biguanide Hydrochloride	Alderley Park rats of the SPF strain (60 males, 60 females per group)	Four groups fed dietary concentrations of 0 ppm, 200 ppm, 1000 ppm, and 2000 ppm, for 122 weeks.	Study terminated at 124 weeks, i.e., due to 80% mortality overall. Accumulative incidence of animals with suspected mammary tumors was comparable between control and treatment groups. Same was true for the number of tumor-bearing animals and the site and incidence of tumors. Non-oncogenic.[17]

Table 14. Carcinogenicity Studies

Ingredient	Animals/Cells	Protocol	Results
Polyhexamethylene Biguanide Hydrochloride	Groups of 5 male C57BI mice	Concentrations of 0, 100, 200, 400, 1200, and 4000 ppm in diet for 7, 14, or 28 days. Immunohistochemical detection of BrdUin mouse liver used to quantify cell proliferatiion in liver endothelial cells. Liver hepatotoxicity assessed by measuring alanine aminotransferase and aspartate aminotransferase in plasma of animals killed	Polyhexamethylene Biguanide Hydrochloride increased cell proliferation in dose-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 ppm and 4000 ppm (after feeding for 28 days) and at 100 ppm and 200 ppm (after feeding for 14 days).[1]
Polyhexamethylene 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/day in males and 0, 14.9, 45.3 and 162.3 mg/kg/ day in females) in diet for 2 years.	Hemangioma (2/64 males and 2/64 females) and hemangiosarcoma (1/64 females) in the liver of animals fed 2000 ppm.[20]

Table 15. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
		Irritation Studies	
Animal Studies			
Polyhexamethylene Biguanide Hydrochloride	5 male New Zealand White rabbits	Test substance (0.5 g, moistened with distilled water) applied to 3 sites on back (dose/cm^2 not stated); sites covered with cotton gauze patch secured with adhesive tape. Patches removed after 3 minutes, 1 h, and 4 h.	Slight edema at 1 h after patch removal and very slight edema at 24 h and 48 h. After 4 h, very slight to well defined erythema; primary irritation index (PII) = 1. Mean value (at 24 h, 48 h, and 72 h) for either erythema and eschar formation or edema formation calculated for each animal tested was ≤ 1. No skin reactions after 7 days. Mild skin irritant.[4]
Polyhexamethylene Biguanide Hydrochloride (96%, as powder)	3 male New Zealand White Rabbits	Test substance (0.5 g moistened with 0.5 ml water) applied under occlusive patch to 3 sites on back of 1 rabbit. Dose per cm^2 not stated. Patches removed after 3 minutes, 1 h, and 4 h. For remaining 2 rabbits, patch remained in place for 4 h.	No irritation after 3-minute or 1-h application. After 4-h exposure, primary irritation index of 1 reported; very slight (at 1 h, 48 h, and 72 h after patch removal) to well-defined (at 4 h and 24 h) erythema observed. Slight edema (at 1h) and very slight edema (at 24 h and 48 h). No reactions at 7 days after patch removal. Mild skin irritant.[1]
25% aqueous Polyhexamethylene Biguanide Hydrochloride	3 female rats (strain not stated)	Test substance applied (dose per cm^2 not stated) under occlusive dressing to intact skin of back for 3 alternating 24-h periods, i.e., each application period followed by 24-h non-treatment period.	Focal ulceration observed after first 24-h application. Reaction increased in severity after 2nd and 3rd applications, by which time there was pronounced edema.[17]
25% aqueous Polyhexamethylene Biguanide Hydrochloride	2 groups of 20 (10 males, 10 females) healthy albino rats of the SPF strain	2 groups received a topical application of test substance to intact skin at doses of 2.5 ml/kg and 5 ml/kg, respectively. Test substance spread over 1 $inch^2$ area; site covered with dressing for 24 h.	Severe skin irritation in all animals.[17]
25% aqueous Polyhexamethylene Biguanide Hydrochloride	Albino guinea pigs (6 test and 4 control) of Porton strain	Both ears treated (patch application; 0.1 ml per ear) with 25% Polyhexamethylene Biguanide Hydrochloride once per day for 3 consecutive days. Next, 0.2 ml of following concentrations (in dimethylformamide) applied to flank (1-cm diameter area): 25% , 12.5%, and 10%	Slight to moderate erythema (irritant effect) on ear at 25%. [4]
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	Groups of 10 (5 males, 5 females per group) rats 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, 60 mg/kg, and 200 mg/kg, respectively, 6 h per day for 30 days (21 applications total).	Slight irritation at 60 mg/kg/day; in most animals, had regressed by end of study. Moderate irritation in all animals at 200 mg/kg/day; in most animals, persisted until end of study. Skin irritation observed was confirmed microscopically and considered test substance-related.[4]

Table 15. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
20% aqueous Polyhexamethylene Biguanide Hydrochloride	9 (3 males, 6 females) New Zealand White rabbits	Test substance applied to 6 rabbits (0.5 ml, under occlusive dressing) for 24 h to ~ 6.25 cm^2 area of intact and abraded skin of the flanks. Similar application to 3 male rabbits; animals then killed at 48 h or 72 h post-application for histopathologic examination of test site.	Moderately irritating to intact skin. Severely irritating to abraded skin.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	6 New Zealand White rabbits	Skin corrosivity test. Applied to intact and abraded skin (dose per cm^2 and duration of application not stated).	Superficial scabbing and erythema around the abrasions. No signs of necrosis at intact skin sites. Non-corrosive.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	6 female albino rabbits	12,000 ppm solution (1 ml) applied to back for 23 h (dose per cm^2 not stated; no occlusion). 21 daily applications.	Non-irritant.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	5 female rats of the Alderley Park strain	Test substance (0.04% active ingredient) applied (0.1 ml; dose per 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 h.	Non-irritant.[17]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	3 rabbits (strain not stated)	Applied to skin for 24 h (dose per cm^2 not stated).	Moderate erythema at 24 h post-application. Completely reversible within 8 days. No edema.[4]
Polyhexamethylene Biguanide Hydrochloride (0.2% in ethanol, 10% in ethanol and 20% [solvent not stated])	4 groups of specific pathogen free (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 (in ethanol), 0.6 mg (0.2% Polyhexamethylene) Biguanide Hydrochloride in ethanol), 6.0 mg (20% Polyhexamethylene Biguanide Hydrochloride) and 30 mg (10% Polyhexamethylene Biguanide Hydrochloride in ethanol).	The highest dose (10% concentration; 30 mg dose) caused noticeable skin irritation in males and females immediately after application. Erythema observed during first few weeks. After 4^{th} week, hyperkeratosis became evident, especially in males. Also, occasionally, there was ulceration extending to the deeper layers of the dermis at the application site.[17]

Table 15. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
Human Studies			
20% aqueous Polyhexamethylene Biguanide Hydrochloride	45 volunteers (17 males, 28 females)	Following concentrations in purified water) applied topically (Finn chamber) for 24 h 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.[4]
Bacterial nanocellulose dressing loaded with 1% w/v sericin and 0.3% w/v Polyhexamethylene Biguanide	105 healthy volunteers	Initially, skin randomly patched with dressings (2 x 2 cm^2 area). After 3 days, new dressings patched onto same area. After an additional 3 days, dressings removed; removal followed by 7- to 10-day non-treatment 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 non-irritating to the skin.[24]
Sensitization Studies			
In Vivo Assay			
Polyhexamethylene Biguanide		Local lymph node assay	Weak sensitizer.[25]
Animal Studies			
20.2% aqueous Polyhexamethylene Biguanide Hydrochloride	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 h. Challenge: occlusive epicutaneous application (24 h) 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 h and 16/20 test animals at 48 hr. Moderate sensitizer.[4]
20.2% Polyhexamethylene Biguanide Hydrochloride (in saline)	Groups of 10 guinea pigs	Guinea pig maximization test. Intradermal induction with 0.15% Polyhexamethylene Biguanide Hydrochloride and topical induction with 20%. Challenge with 20% or 10%	Moderate erythema at 10% and 20% (1 animal per concentration). Non-sensitizer.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	20 Alderly 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 h) and in 15 of 20 animals (at 48 h). Moderate to strong sensitizer.[4]

Table 15. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
20% aqueous Polyhexamethylene Biguanide Hydrochloride	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% Polyhexamethylene Biguanide Hydrochloride. Topical induction and challenge with 20%. 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.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	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 during topical induction (occlusive dressing) for 6 h. Induction repeated for 10 days. Challenge exposures (2 % active ingredient) of 6 h 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 fait erythema at concentrations of 4% (8 of 9 animals) and 2% (3 of 10 animals) active ingredient. 2% active ingredient considered moderate sensitizer.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	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%.[4]

Table 15. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
Human Studies			
20% aqueous Polyhexamethylene Biguanide Hydrochloride	191 volunteers (49 on Panel 1, 114 on Panel 2, and 28 on Panel 3)	During induction, test substance applied (2 x 2 cm patches moistened with 0.5 ml aliquots) for 24 h to dorsal surface of upper arm at concentrations of 2% and 4% active ingredient. Repeated 3 times per week for 10 applications total. Applied at following concentrations during challenge phase: 0.05%, 0.1%, 0.2%, 0.5%, 1% and 2% active ingredient.	Panel 1: At challenge, 8 of 49 subjects (16%) had skin reactions to 2 %, 7 of 49 (14%) with reactions to 1% and 0.5 %, and 2 of 49 (4%) with weak reactions at 0.1%. Panel 2: 18 of 114 subjects (16%) with skin reactions to 0.5% and 7 of 114 (6%) with reactions to 0.2%. 2 other subjects with reactions during non-treatment period following 2% induction, characterized as likely allergic to 2%. Same true for 10 other subjects regarding reactions (described as weak) at late 2% induction. Panel 3: 1 of 28 subjects (3.6%) with reaction to 0.5%. Conclusions: 2% concentration not capable of causing primary skin irritation, but capable of causing skin sensitization humans, which can be elicited at concentrations starting at 0.2 % active ingredient.[4]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	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% x 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. Polyhexamethylene Biguanide Hydrochloride sensitization considered extremely rare.[4,27]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	1975 patients	Multicenter study. Patch testing with 2.5% aqueous (effective concentration = 2.5% x 20% = 0.5%) and 5% aqueous (effective concentration = 5% x 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 0.5% 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, i.e. 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.[4]

Table 15. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
2.5% aqueous Polyhexamethylene Biguanide Hydrochloride	374 patients (multicenter study in United Kingdom)	Patch test (protocol not stated)	2 positive patch test reactions. Data series suggested that baseline frequency of Polyhexamethylene Biguanide Hydrochloride sensitization was very low (0.5%) in United Kingdom. Majority of reactions were weak, and data suggested that Polyhexamethylene Biguanide Hydrochloride may not be a relevant contact allergen.[26]
2.5% aqueous Polyhexamethylene Biguanide Hydrochloride	1154 patients (multicenter study in Germany)	Patch test (protocol not stated)	6 positive patch test reactions. Data series suggested that baseline frequency of Polyhexamethylene Biguanide Hydrochloride sensitization was very low (0.4%) in Germany. Majority of reactions were weak, and data suggested that Polyhexamethylene Biguanide Hydrochloride may not be a relevant contact alldrgen.[26]
2.5% aqueous Polyhexamethylene Biguanide Hydrochloride	1974 patients (multicenter study)	Patch tests (performed in accordance with recommendations of the ICDRG and the DKG)	9 patients (0.5%) with positive patch test reactions. Majority of reactions were weak. No evidence of axillary dermatitis. Occupational exposure considered most probable cause of sensitization.[28]
Phototoxicity/Photosensitization Studies			
Animal Study			
20% aqueous Polyhexamethylene Biguanide Hydrochloride	10 male rats	2 concentrations of test substance (in distilled water) evaluated: 10% (effective concentration = 10% x 20% = 2%) and 25% (25% x 20% = 5%). Each test concentration (0.1 ml) applied to dorsal skin once daily for 4 days. Site irradiated with UVC (black lamp) for 3 h daily.	Very strong irritant potential, but no significant photoirritancy.[17]
Human Study			
20% aqueous Polyhexamethylene Biguanide Hydrochloride	26 male and female subjects	Diluted test substance (1:20 in water; effective concentration = 1%) evaluated. Patches (20 x 20 mm square of Webril affixed to 40 x 40 mm adhesive square) moistened with 0.4 ml of the test substance. Patches applied to upper arm for 24 h, 3 times per week for 4 successive weeks. Immediately after patch removal, sites exposed to direct rays of mid-day sun for 1 h. Challenge application at week 6.	Test substance (at 1%) essentially non-irritating and did not induce sensitization, phototoxicity, or photoallergenicity.[17]

Table 16. Ocular Irritation Studies

Ingredient	Number of Animals	Test Protocol	Results
Polyhexamethylene Biguanide Hydrochloride (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.[4]
Polyhexamethylene Biguanide Hydrochloride (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 post-instillation and considered irreversible reactions. Test substance was corrosive to rabbit eye.[4]
25% aqueous Polyhexamethylene Biguanide Hydrochloride	3 rabbits (strain not stated).	Single instillation (volume not stated). 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.[17]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	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.[4]
20% Polyhexamethylene Biguanide Hydrochloride	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.[17]
20% Polyhexamethylene Biguanide Hydrochloride	3 rabbits (strain not stated)	Test substance (diluted to 0.04% active ingredient; 0.1 ml) instilled into eyes	No immediate or delayed irritant effects observed.[17]
20% aqueous Polyhexamethylene Biguanide Hydrochloride	Donated human eyes (41) not suitable for clinical use 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.[29]

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