Safety Assessment of Polyaminopropyl Biguanide as Used in Cosmetics

Status: Draft Report for Panel Review
Release Date: March 17, 2017
Panel Date: April 10-11, 2017

The 2017 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst., Bart Heldreth, Ph.D., Chemist, and Ivan Boyer, Ph.D., Toxicologist.

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: March 17, 2017
Subject: Polyaminopropyl Biguanide

A Scientific Literature Review (SLR) on Polyaminopropyl Biguanide was issued by the Cosmetic Ingredient Review (CIR) on February 13, 2017, and the Draft Report (*polyam042017rep*) is being reviewed at this Panel meeting. The ingredient name, Polyaminopropyl Biguanide, refers to the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units (polyhexamethylene biguanide hydrochloride (PHMB HCl)). Frustratingly, the ingredient name, Polyaminopropyl Biguanide, contains none of the chemical, polyaminopropyl biguanide (a 3 carbon chain in each monomeric repeat unit; PAPB), but instead applies exclusively to polyhexamethylene biguanide (a 6 carbon chain in each monomeric repeat unit; always supplied as the hydrochloride salt). Indeed, the chemical, PAPB, is not a cosmetic ingredient. Accordingly, throughout the report, the ingredient name Polyaminopropyl Biguanide will be used exclusively even though the chemical under evaluation is polyhexamethylene biguanide hydrochloride (PHMB HCl). The Ingredient Nomenclature Committee (INC) has recently (after the SLR was drafted) modified the ingredient monograph for Polyaminopropyl Biguanide to clarify that this ingredient name refers only to the chemical PHMB HCl.

The following ingredient data that were submitted by the Council have been added to the Draft Report: Use concentration data (*polyam042017data1 and polyam042017data1*), Supplier comments on the identity of Polyaminopropyl Biguanide (*polyam042017data2*), a Cosmetics Europe Dossier on the safety of Polyaminopropyl Biguanide (*polyam042017data3 and polyam042017data3a*), and the revised *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*) monograph on Polyaminopropyl Biguanide (*polyam042017data4*). Comments that were received from the Council (*polyam042017pcpc*) have also been incorporated.

The Council also provided CIR with the following 3 publications relating to polyhexamethylene guanidine phosphate (PHMG phosphate, humidifier disinfectant) inhalation exposure and lung injury in humans: *An Analysis of a Humidifier Disinfectant Case From a Toxicological Perspective* (Park, 2016), *Humidifier Disinfectants are a Cause of Lung Injury Among Adults in South Korea: A Community-Based Case-Control Study* (Park et al., 2016), and *Humidifier Disinfectant-Associated Children's Interstitial Lung Disease* (Kim et al., 2014). These studies are not included in the Draft Report because Polyaminopropyl Biguanide and PHMG phosphate are different chemicals and the report already contains inhalation toxicity data on Polyaminopropyl Biguanide. Furthermore, a statement on the relevance of the 3 publications to the safety assessment of Polyaminopropyl Biguanide was not provided. The Panel will need to determine whether or not additional inhalation toxicity data are needed.

Included in this package for your review is the Draft Report (*polyam042017rep*), the CIR report history (*polyam042017hist*), Literature search strategy (*polyam042017strat*), Ingredient data profile (*polyam042017prof*), 2017 FDA VCRP data (*polyam042017FDA*), Use concentration data received from the Council (*polyam042017data1 and polyam042017data1*), Cosmetics Europe Dossier received from the Council (*polyam042017data3 and polyam042017data3a*), *the* revised *Dictionary* monograph (*polyam042017data4*), and Comments that were received from the Council (*polyam042017pcpc*).

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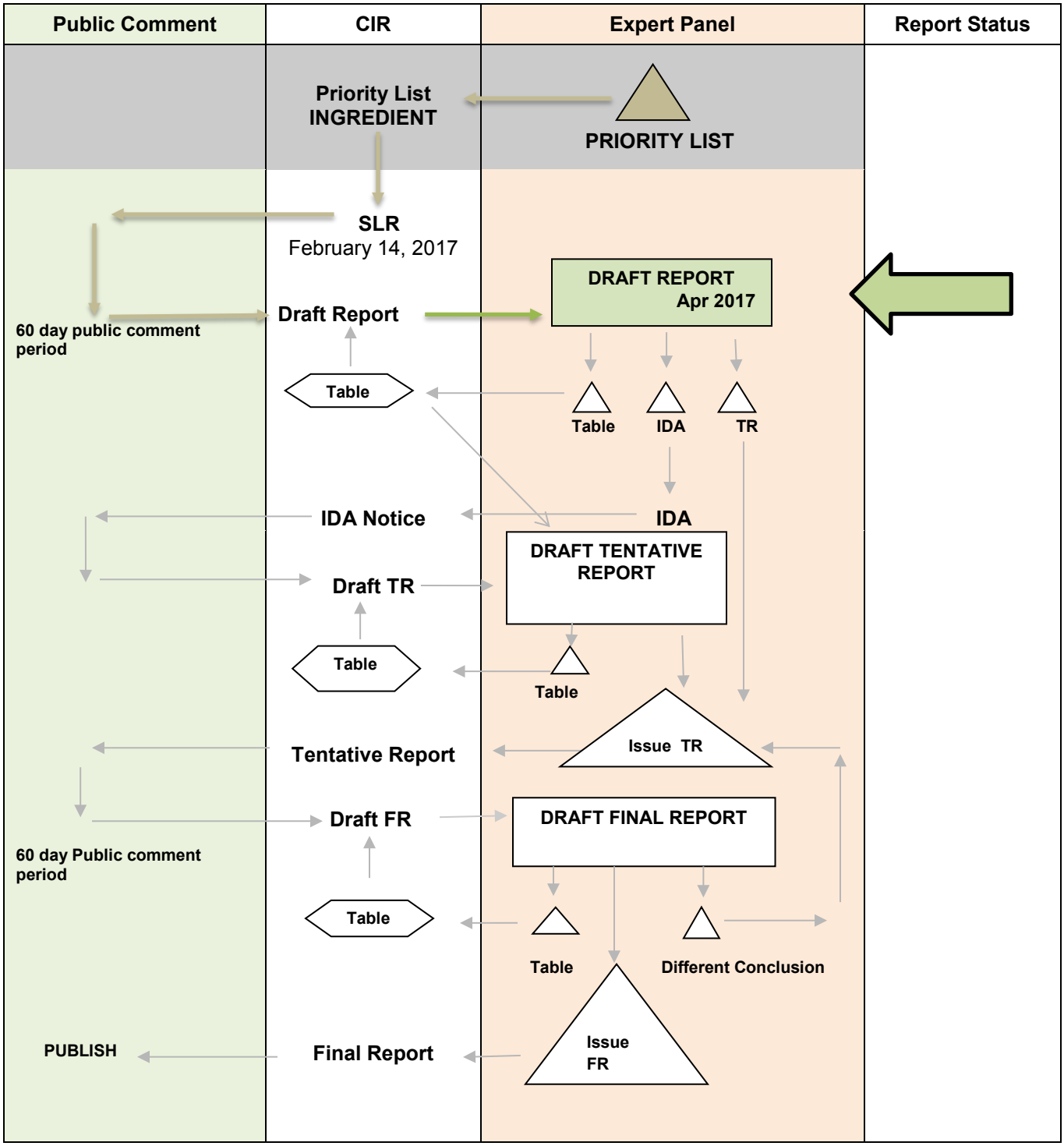
At this meeting, the Panel needs to determine whether or not an insufficient data announcement needs to be issued, or whether a tentative report with a safe as used, safe with qualifications, or unsafe conclusion should be issued.

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Polyaminopropyl Biguanide

MEETING April 2017



CIR History of:

Poloyaminopropyl Biguanide

A Scientific Literature Review (SLR) on Polyaminopropyl Biguanide was issued on February 13, 2017.

Draft Report, Teams/Panel: April 10-11, 2017

The following ingredient data that were submitted by the Council have been added to the Draft Report: Use concentration data, Supplier comments on the identity of Polyaminopropyl Biguanide, and a Cosmetics Europe Dossier on the safety of Polyaminopropyl Biguanide. Comments that were received from the Council (*polyam042017pcpc*) have also been incorporated.

Polyaminopropyl Biguanide Data Profile for April 10th-11th, 2017 Panel – Wilbur Johnson

			Dermal Penetration			Nail Penetration	Penetration Enhancement	ADME					Acute Toxicity			Short-Term Toxicity	Sub-Chronic Toxicity	Chronic Toxicity	DART		Genotoxicity	Carcinogenicity	Other Relevant Studies		Dermal Irritation*	Dermal Sensitization*/ Phototoxicity*		Ocular Irritation *		Clinical Studies	Case Reports		Epidemiology Studies
			In Vivo -Animal	In Vitro-Human	In Vivo-Human	In Vitro-Human	In Vitro-Animal	In Vitro-Human Dermal	Animal-Dermal	Animal-Oral	Animal-IV	Human-Oral	Animal-Dermal	Animal-Oral	Animal-Inhalation	Animal	Animal	Animal	In Vitro	In Vivo	In Vitro/In Vivo	In Vivo/In Vivo	In Vitro	In Vivo-Animal	Animal/Human	Animal	Human	In Vitro	Animal/Human	Human-Dermal/Oral	Human-Dermal	Human-Oral	Human
Polyaminopropyl Biguanide				**X**						**X**			**X**	**X**	**X**	**X**	**X**	**X**		**X**	**X/X**	**X/X**			**X/X**	**X/X**	**X/X**		**X/X**	X/0	X		

X = data; 0 = no data*

[Polyaminopropyl Biguanide (11/09/2016 & 11/14/2016; Updated on 3/7/2017)]

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	FEMA	ECETOC
Polyaminopropyl Biguanide	133029-32-0 32289-58-0	1/1	18/162	3/126	3/11	No	Yes	No Dossier	No	No	No	No	No	No	No	No	No	No
Polyhexamethylene Biguanide	28757-47-3	1/1	8/84	13/370	4/99	no	Yes	No Dossier	No	No	Yes	Yes	1/19	1/4	0/2	No	No	No

Search Strategy

[*document search strategy used for SciFinder, PubMed, and Toxnet*]

[*identify total # of hits /# hits that were useful or examined for usefulness*]

LINKS

InfoBase (self-reminder that this info has been accessed; not a public website) - http://www.personalcarecouncil.org/science-safety/line-infobase
ScfFinder (usually a combined search for all ingredients in report; list # of this/# useful) - https://scifinder.cas.org/scifinder
PubMed (usually a combined search for all ingredients in report; list # of this/# useful) - http://www.ncbi.nlm.nih.gov/pubmed
Toxnet databases (usually a combined search for all ingredients in report; list # of this/# useful) – https://toxnet.nlm.nih.gov/ (includes Toxline; HSDB; ChemIDPlus; DAR; IRIS; CCRIS; CPDB; GENE-TOX)

FDA databases – http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (CFR); then,
list of all databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm; then,
http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true (EAFUS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm (GRAS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm (SCOGS database);
http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives (indirect food additives list);
http://www.fda.gov/Drugs/InformationOnDrugs/default.htm (drug approvals and database);
http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf (OTC ingredient list);
http://www.accessdata.fda.gov/scripts/cder/iig/ (inactive ingredients approved for drugs)

EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - http://ec.europa.eu/growth/tools-databases/cosing/
ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/
NTIS (National Technical Information Service) - http://www.ntis.gov/
NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/ (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - http://www.ecetoc.org/

Botanical Websites, if applicable
Dr. Duke's https://phytochem.nal.usda.gov/phytochem/search
Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
Sigma Aldrich plant profiler http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html

Fragrance Websites, if applicable
IFRA (International Fragrance Association) – http://www.ifraorg.org/

RIFM (the Research Institute for Fragrance Materials) should be contacted

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INTRODUCTION

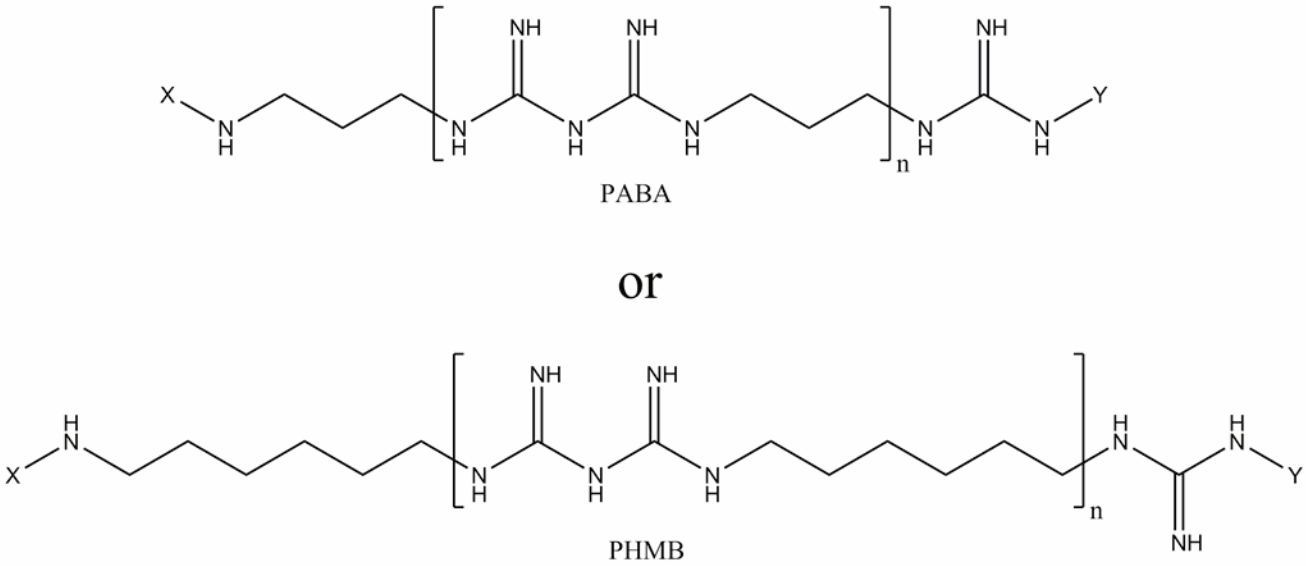
The safety of Polyaminopropyl Biguanide (International Nomenclature of Cosmetic Ingredients [INCI] name[1]) as used as a preservative in cosmetics is reviewed in this assessment. The ingredient name, Polyaminopropyl Biguanide, refers to the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units (polyhexamethylene biguanide hydrochloride (PHMB HCl)). However, the ingredient name, Polyaminopropyl Biguanide, contains none of the chemical, polyaminopropyl biguanide (a 3 carbon chain in each monomeric repeat unit; PAPB), but instead applies exclusively to polyhexamethylene biguanide (a 6 carbon chain in each monomeric repeat unit; always supplied as the hydrochloride salt). Indeed, the chemical, PAPB, is not a cosmetic ingredient. Accordingly, throughout this report, the INCI name Polyaminopropyl Biguanide will be used exclusively even though the chemical under evaluation is polyhexamethylene biguanide hydrochloride (PHMB HCl). 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 ended on March 10, 2017.[2]

Additionally, 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.[3]

CHEMISTRY

Definition and General Characterization

Polyaminopropyl Biguanide is the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units (polyhexamethylene biguanide (PHMB HCl), and the definition and idealized structures are also presented in Table 1.[1]



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 only for the chemical, PHMB HCl.

However, the current wINCI monograph for Polyaminopropyl Biguanide has recently been updated to define this ingredient as the chemical, PHMB HCl, as depicted in the structure and in the CAS File corresponding to the CAS No. in the wINCI monograph.[1]

Comments on the identity of Polyaminopropyl Biguanide were received from a chemical supplier, wherein it is stated that effectively all PHMB is Poly (hexamethylene Biguanide) HCl (i.e., C6 alkyl chains linked by biguanide groups), and no propyl biguanide groups are present (this in an artifact of the INCI name, as the polymer repeating unit has been arbitrarily chosen as the middle of the C6 alkyl chain).[4]

Chemical and Physical Properties

Polyaminopropyl Biguanide is 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.[5] Chemical and physical properties are summarized in Table 2.

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Method of Manufacture

One of the current methods for manufacturing Polyaminopropyl Biguanide is via the polycondensation of sodium dicyanamide and hexamethylenediamine.[6]

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.[2]

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).[2]

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.[7] 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.[8]

According to 2017 VCRP data, Polyaminopropyl Biguanide is being used in 147 cosmetic products, mostly leave-on products.[7] 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).[8]

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.[9,10,11,12] 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. [9,10]

The SCCS originally 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%.[5] 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 ended on March 10, 2017.[2]

Polyaminopropyl Biguanide 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%.[2,13] Additionally, Polyaminopropyl Biguanide is classified as CMR 2 (Carc. 2) according to the Commission Regulation (EU) No. 944/2013. CMR substances are classified as carcinogenic, mutagenic, or toxic for reproduction. A substance is placed in carcinogen Category 2 (Carc. 2, suspected human carcinogens) when the evidence obtained from human and/or animal studies is not sufficiently convincing to place the substance in Category 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.[2] 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.[13] 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

Polyaminopropyl Biguanide has been reported to be the most frequently used antiseptic in traumatic and orthopedic surgery.[14] According to another source, Polyaminopropyl Biguanide has the following uses: fungicide, algicide, sanitizer in swimming pools, preservative for cut flowers, materials preservative, bacteriostat in industrial processes, and water systems, and hard surface disinfectant (food and non-food contact surfaces.[3]

Polyaminopropyl Biguanide is a broad-spectrum antimicrobial agent used in a variety of products including contact lens cleaning solutions, skin disinfectant solutions, and wound dressings.[15] Solid wound dressings are composed of various synthetically or naturally derived materials, and typically contain added antimicrobials such as silver, bismuth, chlorhexidine, bacitracin, or Polyaminopropyl 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.[16]

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

TOXICOKINETIC STUDIES

Dermal Penetration

The dermal penetration studies summarized below are presented in Table 4.

In Vitro

In one study, skin penetration experiments were performed using both rat (skin disks in solutions; 5-day equilibration phase) and human skin (receptor fluid [in diffusion cell] collected up to 15 days) in vitro. At concentrations of 0.4%, 1.4%, 5%, and 20% Polyaminopropyl Biguanide, 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, absorption rates (ng/cm^2/h) through rat whole skin were 131, 3695, and 11940, respectively. Another study involved the application of Polyaminopropyl Biguanide (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 solutions (0.1% aqueous micellar solution (lowest concentration applied in any study) and a slightly higher 0.3% in oil-in-water emulsion) were applied to human split-thickness skin in a 2-part dermal penetration study. In Part 1 of this study, penetration of the 0.1% aqueous solution and 0.3% in oil-in-water emulsion, respectively was determined directly after the 24-h exposure period. In Part 2, 24 h exposure to the 0.1% aqueous solution and to 0.3% in an oil-in-water emulsion, respectively, was followed by an additional 72-h period to determine if the emulsion moved from the skin to the receptor fluid. During the 72-h period, most of the radioactivity in the skin remained in the skin. Study results indicated that the value for the absorption of Polyaminopropyl Biguanide through the skin was 4.09%.[5]

Absorption, Distribution, Metabolism, and Excretion

The toxicokinetic studies (oral exposure) summarized below are presented in Table 5.

Animal

Oral

In rats, the principal route of excretion of radioactivity from radiolabeled Polyaminopropyl Biguanide 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 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.[5,18,19]

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The acute toxicity data summarized below 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; but hemorrhage of dermal capillaries at the application site was observed. In an acute dermal toxicity study on 20% aqueous Polyaminopropyl Biguanide on rabbits, the LD_{50} was reported to be > 400 mg/kg.[5,18]

Oral

An LD_{50} of > 1000 mg/kg was reported for rats dosed orally with aqueous solutions (up to 25% aqueous) of Polyaminopropyl Biguanide. A median lethal dose of 25.6 mg/kg was reported for rats dosed orally with a solution of 0.4% Polyaminopropyl Biguanide.[5,17,18,20,21]

Risk Assessment

The EPA conducted a screening-level acute dietary human health risk assessment for Polyaminopropyl Biguanide food uses.[3] 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 = 0.2 mg/kg/day), 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 (mostly 4-h exposures) in which rats were exposed to Polyaminopropyl Biguanide 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 at concentrations up to 208 mg/m^3.[5]

Short-Term Toxicity Studies

The short-term dermal, oral, and inhalation toxicity studies summarized below 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 Polyaminopropyl Biguanide over a 60-day period. Similar results were reported for rats after dermal applications of Polyaminopropyl Biguanide 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 was applied.[5,18]

Oral

A lowest-observed-adverse-effect-level (LOAEL) of 0.1 mg/ml for Polyaminopropyl Biguanide was reported in 28-day oral toxicity studies involving rats.[5,18,20] In a 60-day oral toxicity study involving rats, mild toxicity in the liver or kidneys (at microscopic examination) was observed at daily doses of 2 mg/kg, 6 mg/kg, and 32 mg/kg (highest dose).

Inhalation

In 21-day and 28-day inhalation toxicity studies on Polyaminopropyl Biguanide involving rats, no-observed-adverse-effect-concentrations (NOAECs) equal to 0.025 mg/m^3 and 0.0239 mg/m^3, respectively, were reported. The greatest exposure was 26 mg/m^3 for 5 days per week (6 h per day) [5]

Subchronic Toxicity Studies

The subchronic oral toxicity studies summarized below are presented in Table 10.

Oral

The following results were reported in 90-day oral toxicity studies on Polyaminopropyl Biguanide 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, 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 at concentrations up to 11000 ppm in the diet for 90 days.[5,18]

Chronic Toxicity Studies

The chronic dermal and oral toxicity studies summarized below are presented in Table 11.

Dermal

In an 80-week chronic toxicity study involving mice (dermal applications 5 days/week), a mortality rate of 75% was reported for the highest dose group (10% Polyaminopropyl Biguanide, 30 mg dose). 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.[18]

Oral

In a 104-week oral toxicity study, a NOAEL of 2000 ppm (highest concentration fed in diet) was reported for Polyaminopropyl Biguanide. This concentration corresponded to a daily dose of 36 mg/kg/day in male rats. A no-observed-effect-level (NOEL) of 200 ppm for histopathologic changes was reported in a 122-week oral toxicity study involving rats fed Polyaminopropyl Biguanide at concentrations up to 2000 ppm in the diet. In a study involving mice, feeding with Polyaminopropyl Biguanide (concentrations up to 1000 ppm in diet) for 97 weeks did not cause any macroscopic changes in tissues examined. A NOAEC of 1500 ppm for Polyaminopropyl Biguanide 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 produced dose-related hepatotoxicity and nephrosis.[5,18,21]

Risk Assessment

In the chronic oral toxicity study that is summarized in Table 11, Polyaminopropyl Biguanide (20.2% aqueous) was administered in the 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). The NOAEL of 36 mg/kg/day, systemic exposure dose (SED) of 0.0666 mg/kg/day, and the assumption for dermal penetration of 7.65% were used to calculate a margin of safety (MOS) for Polyaminopropyl Biguanide (MOS = 46).[5] 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 (assumption for dermal absorption = 4.09%) are 258 and 227 (i.e., MOS lower when additional exposure from non-cosmetic use is incorporated).[2]

In an EPA human health risk assessment, residential-handler and post-application exposure scenarios relating to pesticide (including Polyaminopropyl Biguanide) application were assessed using surrogate exposure data, maximum application rates (on product label), and standard assumptions.[3] 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 (oral NOAEL of 20 mg/kg/day used in calculation) 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.[3] 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.[3]

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).[3]

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

The developmental and reproductive toxicity studies summarized below are presented in Table 12.

In oral reproductive and developmental toxicity studies on Polyaminopropyl Biguanide (in the diet) 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 (6 h/day, 5 days/week), 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. Polyaminopropyl Biguanide 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.[5,2,18,21]

GENOTOXICITY STUDIES

The genotoxicity studies (in vitro and in vivo) summarized below are presented in Table 13.

In the Ames test, Polyaminopropyl Biguanide 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 was weakly genotoxic in strain 1538 without metabolic activation. Polyaminopropyl Biguanide was non-genotoxic in the mouse

lymphoma assay at concentrations up to 2000 µg/ml with and without metabolic activation, or 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 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.[5]

CARCINOGENICITY STUDIES

The carcinogenicity studies (in vitro, dermal and oral) summarized below are presented in Table 14.

In Vitro

Polyaminopropyl Biguanide was evaluated at concentrations up to 3000 µg/ml in the cell transformation assay (using baby hamster kidney fibroblasts); there was no difference in the number of transformed cell colonies between test and negative control cultures. In another assay involving RAW 264.7 mouse macrophages, Polyaminopropyl Biguanide, tested at concentrations up to 1 ppm, had no direct effect on liver cell proliferation and did not potentiate cell proliferation induced by activated macrophages.[2,5]

Dermal

Polyaminopropyl Biguanide was classified as a hepatic tumorigen in mice, i.e., at the highest dose (30 mg of 10% Polyaminopropyl Biguanide 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. The mortality incidence in other dose groups was not reported.[5,18]

Oral

A statistically significant increase in the incidence of hemangiosarcomas and hemangiomas was reported for only male mice that received Polyaminopropyl Biguanide at a dietary concentration of 4000 ppm daily for 2 years. In a 97-week study in which mice were fed Polyaminopropyl Biguanide 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 in the diet. Polyaminopropyl Biguanide 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 at a dietary concentration of 2000 ppm for 2 years.[5,2,18,22]

OTHER RELEVANT STUDIES

Effect on Lung Cells

A study was performed to elucidate the inflammatory responses and its mechanisms induced by Polyaminopropyl Biguanide in lung cells.[23] A549 cells that were exposed to Polyaminopropyl Biguanide showed concentration-dependent (0 to 80 µg/mL) decreased viability, significant reactive oxygen species (ROS) generation (at 20 µg/mL), inflammatory cytokine secretion (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 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 was completely suppressed by a NF-κB inhibitor, but not by a ROS scavenger. The authors suggested that Polyaminopropyl Biguanide induces inflammatory responses via the NF-κB signaling pathway.

Cytotoxicity and Antimicrobial Activity

Polyhexamethylene biguanide was compared to the (structurally) closely related Polyaminopropyl Biguanide (not a cosmetic ingredient) with respect to antiseptic efficacy and cytotoxicity in vitro.[24] Antimicrobial efficacy tests were performed via determination of the minimum bactericidal concentration (MBC). Polyhexamethylene Biguanide exhibited high antimicrobial activity against *Staphylococcus aureus* and *Echerichia 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 polyhexamethylene biguanide 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/v and at concentrations ranging from 0.25% to 3% v/v. Cultures were incubated for up to 72 h. For all tested concentrations, Polyaminopropyl Biguanide 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 %. PAPB 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% PAPB. After 72 h, only a weak cytotoxic effect on L929 cell at 0.05% and 0.1% PAPB could be observed, while, for HaCaT cells, concentrations up to 0.1% were classified as non-cytotoxic. However, concentrations $\geq$ 0.25% PAPB were highly cytotoxic to cells of both cell lines after 24 h of incubation. When compared directly, PAPB consistently resulted in a significantly higher cell survival rate than Polyaminopropyl Biguanide, irrespective of concentration and incubation time ($P \leq 0.0006$).[24]

Epigenetic Effects

It has been proposed that Polyaminopropyl Biguanide be classified as a category 3 carcinogen though it is not genotoxic.[25] It has been hypothesized that Polyaminopropyl Biguanide 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 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 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 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 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 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, 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 Biguanidehas been determined and appeared to not be significantly affected. Taken together, the data indicate that Polyaminopropyl Biguanide 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.[25]

DERMAL IRRITATION AND SENSITZATION STUDIES

The skin irritation, sensitization, and phototoxicity/photosensitization studies summarized below are presented in Table 15.

Irritation

Polyaminopropyl Biguanide (single 4-h application) was classified as a mild skin irritant in rabbits. Single applications (24 h) of 20% aqueous Polyaminopropyl Biguanide to rabbits indicates that this compound is non-corrosive,

moderately irritating to intact skin, and severely irritating to abraded skin. Repeated applications of Polyaminopropyl Biguanide (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, at dosages of 2.5 ml/kg and 5 ml/kg. Polyaminopropyl Biguanide (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 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 for 3 days. In a study involving mice, the highest dose of Polyaminopropyl Biguanide (10% concentration in ethanol, 30 mg dose) caused hyperkeratosis and, occasionally, ulceration extending into the dermis when applied repeatedly for 80 weeks. Polyaminopropyl Biguanide (up to 1.5% active) was not classified as a primary skin irritant when applied for 24 h to the skin of human subjects.[2,5,18,26]

Sensitization

Results were positive for Polyaminopropyl Biguanide in the local lymph node assay. In maximization tests, moderate skin sensitization was observed in guinea pigs challenged with Polyaminopropyl Biguanide (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 (20.2% active ingredient). In another guinea pig maximization test, Polyaminopropyl Biguanide (10% or 20%) was classified as a non-sensitizer. In Buehler sensitization tests, Polyaminopropyl Biguanide (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 (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 (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.[5,27,28,29,30,31,32]

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 at concentrations of 2% and 5%. When tested at a concentration of 1% in 26 subjects, Polyaminopropyl Biguanide was essentially non-irritating and did not induce sensitization, phototoxicity, or photoallergenicity.[18]

OCULAR IRRITATION STUDIES

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

Undiluted Polyaminopropyl Biguanide was a severe ocular irritant/corrosive agent when instilled into the rabbit eye. The instillation of 25% aqueous Polyaminopropyl Biguanide into the eyes of rabbits resulted in severe inflammation and corneal damage in unrinsed eyes and slight inflammation in rinsed eyes. Moderate and mild ocular irritation were observed in unrinsed and rinsed rabbit eyes, respectively, after 20% aqueous Polyaminopropyl Biguanide was instilled. In another study involving rabbits, the instillation of Polyaminopropyl Biguanide (20% aqueous) into the eyes induced slight inflammation, but no corneal ulceration. Ocular irritation was not observed when Polyaminopropyl Biguanide (0.04% active ingredient) was instilled into the eyes of rabbits. In a study in which 20% aqueous Polyaminopropyl Biguanide (100 µl) 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.[5,18,33]

CLINICAL STUDIES

The patient multicenter studies summarized below are presented in the Human Sensitization Studies 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.[28,29,30,32]

Case Reports

A male patient had a history of angioedema and pruritus after using wet wipes.[14] Patch test results for an ingredient of the wipes, Polyaminopropyl Biguanide (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.[34] Polyaminopropyl Biguanide, 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 (with a papulovesicular reaction, extending outside of the test chamber) were observed; +? and + reactions to 2% Polyaminopropyl Biguanide were observed on days 2 and 4, respectively. No reactions to 0.2% Polyaminopropyl Biguanide were observed.

Two cases of severe anaphylaxis were reported following contact of a surgical wound with a hospital disinfectant containing 0.2 % Polyaminopropyl Biguanide.[17,35]

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 a disinfectant.[17,36]

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.[37]

Other Clinical Reports

Based on medical surveillance information, obtained between 2004 and 2007, from employees who came in contact with Polyaminopropyl Biguanide in the workplace, no cases of skin sensitization to this chemical were reported.[5] 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 was well-tolerated and there were only two cases of transient local skin erythema.[38] Four of 28 patients were excluded from a cohort study due to adverse effects related to a Polyaminopropyl Biguanide dressing.[39]

Reportedly, the application of very high doses of Polyaminopropyl Biguanide can trigger fever and a generalized exanthema.[21]

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 polyhexamethylene biguanide hydrochloride (PHMB HCl).

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 2017 VCRP data, Polyaminopropyl Biguanide is being used in 147 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 safety of Polyaminopropyl Biguanide 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.

Polyaminopropyl Biguanide (0.1% aqueous micellar solution (lowest concentration applied in any study) and slightly higher 0.3% concentration in oil-in-water emulsion) were applied to human split-thickness skin in a dermal penetration study). In Part 1 of the study, penetration of the 0.1% aqueous solution and 0.3% in an oil-in-water emulsion, respectively was determined directly after the 24-h exposure period. In Part 2, 24 h of exposure to the 0.1% aqueous solution and 0.3% in oil-in-water emulsion, respectively, was followed by an additional 72-h period to determine if the emulsion moved from the skin to the receptor fluid. During the 72-h period, most of radioactivity in the skin remained in the skin. Study results indicated that the value for the absorption of Polyaminopropyl Biguanide through the skin was 4.09%.

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

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

An LC_{50} of > 0.36 mg/l was reported in acute inhalation toxicity studies in which rats were exposed to Polyaminopropyl Biguanide 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 at concentrations up to 208 mg/m^3.

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

In a 60-day dermal toxicity study, there were no mortalities or signs of systemic toxicity in rats administered a 0.4% solution of Polyaminopropyl Biguanide. Similar results were reported for rats after dermal applications of Polyaminopropyl Biguanide at doses up to 200 mg/kg daily over a 30-day period (21 applications total; 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 was applied.

A LOAEL of 0.1 mg/ml for Polyaminopropyl Biguanide was reported in 28-day oral toxicity studies involving rats.

In 21-day and 28-day inhalation toxicity studies on Polyaminopropyl Biguanide involving rats, NOAEL values of 0.025 mg/m^3 and 0.0239 mg/m^3, respectively, were reported. In a 60-day oral toxicity study involving rats, mild toxicity in the liver or kidneys (at microscopic examination) was observed at daily doses of 2 mg/kg, 6 mg/kg, and 32 mg/kg (highest dose).

In 90-day toxicity studies on rats and mice, 4000 to 5000 ppm Polyaminopropyl Biguanide 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 90-day study, 20% Polyaminopropyl Biguanide in drinking water yielded no treatment-related macroscopic findings in rats. A NOAEL of 5500 ppm was reported for Beagle dogs fed up to11000 ppm Polyaminopropyl Biguanide 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, 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. 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 at concentrations up to 2000 ppm in the diet. In a study involving mice, feeding with Polyaminopropyl Biguanide (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 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 produced dose-related hepatotoxicity and nephrosis.

In oral reproductive and developmental toxicity studies on Polyaminopropyl Biguanide involving rats, NOAEL values of 1000 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 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 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 was weakly genotoxic in strain 1538 without metabolic activation. Polyaminopropyl Biguanide 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 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 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, 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 did not exhibit clear and remarkable epigenetic properties.

Polyaminopropyl Biguanide was classified as a hepatic tumorigen in mice, i.e., at the highest dose (30 mg of 10% Polyaminopropyl Biguanide 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 at a dietary concentration of 4000 ppm daily for 2 years. In a 97-week study in which mice were fed Polyaminopropyl Biguanide 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 in the diet. Polyaminopropyl Biguanide 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 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 consistently resulted in a significantly higher cell survival rate (i.e., less cytotoxicity) than Polyaminopropyl Biguanide, irrespective of concentration and incubation time ($P \leq 0.0006$).

The results of animal studies indicate that the skin irritation potential of Polyaminopropyl Biguanide may be concentration-dependent as well as dependent upon the duration of application. For example, the skin irritation potential of Polyaminopropyl Biguanide (single 4-h application) was classified as a mild skin irritant in rabbits. Single applications (24 h) of 20% aqueous Polyaminopropyl Biguanide to rabbits were non-corrosive, moderately irritating to intact skin, and severely irritating to abraded skin. Repeated applications of Polyaminopropyl Biguanide (12,000 ppm) to the skin of rabbits for 21 days were classified as non-irritating. Polyaminopropyl Biguanide (up to 1.5% active) was not classified as a primary skin irritant when applied for 24 h to the skin of human subjects. In a human repeated insult patch test (HRIPT, 191 subjects), it was determined that Polyaminopropyl Biguanide (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.

Positive results were reported for Polyaminopropyl Biguanide 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 (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 (20.2% active ingredient). In another guinea pig maximization test, Polyaminopropyl Biguanide (10% or 20%) was classified as a non-sensitizer.

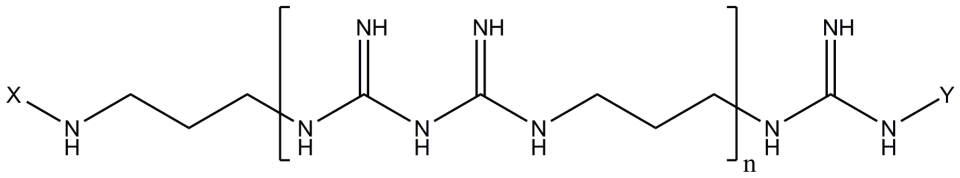
Very strong irritation potential, but no significant photoirritancy, was reported in a study in which male rats were tested with Polyaminopropyl Biguanide at concentrations of 2% and 5%. When tested at a concentration of 1%, in 26 subjects, Polyaminopropyl Biguanide was essentially non-irritating and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

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

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

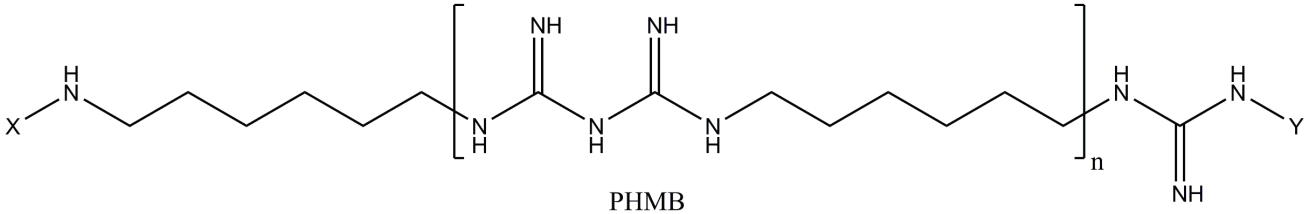
Table 1. **Definition, idealized structures, and function of the ingredient in this safety assessment.**[1; [CIR Staff]]

Ingredient CAS No.	Definition & Idealized Structures	Function
Polyaminopropyl Biguanide 32289-58-0 [PHMB HCl] [27083-27-8 (PHMB HCl)] [28757-47-3 (PHMB)]	Polyaminopropyl Biguanide is the organic compound that conforms to the formula. [Polyaminopropyl Biguanide is the hydrochloride salt of an amino polymer comprising hexyl biguanide repeat units (polyhexamethylene biguanide (PHMB HCl).]	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])]) and/or color	pale yellow powder	[2]
average molecular weight (Daltons [Da])	3686-4216. Molecular weight distribution in commercially used mixture: 6% is < 500, 14.1% is between 500 and 1000, and 75.8% is > 1000	[2]
water solubility (g/100 ml)	41 ± 1 %	[2]
other solubility (g/100 ml)	ethanol: 0.5 ± 0.08% methanol: 41 ± 1 %	[2]
relative density (at 20 ± 0.5°C)	1.20 ± 0.0025	[2]
melting point (°C)	78.9-136.3	[2]
boiling point (°C)	decomposes at 205-210 before boiling	[2]
vapor pressure (Pa at 20°C)	1.32×10^{-7}	[2]
log P_{ow} (at 25 ± 1°C)	-2.3	[2]
UV absorption (λ) (nm)	236	[2]

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

	# of Uses[7]	*Max Conc of Use (%)*[8]
	Polyaminopropyl Biguanide	
Totals*	**147**	**0.000002-0.5**
Duration of Use		
Leave-On	*102*	*0.000002-0.5*
Rinse-Off	*45*	*0.00025-0.5*
Diluted for (Bath) Use	*NR*	*NR*
Exposure Type		
Eye Area	28	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	116	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]- Polyaminopropyl Biguanide, 20.2% aqueous; specific activity = 0.88 mCi/ml)	Various concentrations applied to human skin (epidermis from abdominal skin) in diffusion cell. (dose volume = 1 ml). Receptor fluid samples collected daily for up to 15 days. Also, uptake experiment whereby 2 cm^2 rat skin disks (whole skin from flank and dorsum of male and female Wistar-derived, Alderley-Park rats) bathed in different concentrations; 5-day equilibration phase.	At concentrations of 0.4%, 1.4%, 5%, and 20%, absorption rates (ng/cm^2/h) through human epidermis were 8.13, 22.8, 350, and 1005, respectively. At concentrations of 0.4%, 20% (early phase [not defined]), and 20% (late phase [not defined]) [^{14}C]- Polyaminopropyl Biguanide, absorption rates (ng/cm^2/h) through rat whole skin were 131, 3695, and 11940, respectively.[5]
[^{14}C]- Polyaminopropyl Biguanide (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).[5]
[^{14}C]- Polyaminopropyl Biguanide (0.1% w/w in aqueous micellar solution); [^{14}C]- Polyaminopropyl Biguanide (0.3 % w/w in oil-in-water emulsion)	0.1% in aqueous micellar solution and 0.3% in oil-in-water emulsion, respectively, applied (24-h exposure study) to human split-thickness skin from 4 donors (dose volume = 200 µl/cm^2; application rate ≈ 2 mg/cm^2) in diffusion cell. Penetration was determined directly after exposure.	Following results relate to 0.1% in aqueous micellar solution and 0.3% oil-in-water emulsion, respectively: 48.3% and 52.35% of radioactivity, respectively, removed during washing procedure. At 24 h, absorbed dose (receptor fluid + receptor wash) 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).[2]
[^{14}C]- Polyaminopropyl Biguanide (0.1% w/w in aqueous micellar solution); [^{14}C]- Polyaminopropyl Biguanide (0.3 % w/w in oil-in-water emulsion)	0.1% in aqueous micellar solution and 0.3% oil-in-water emulsion, respectively, applied (24 h of exposure, followed by 72-h period to determine if emulsion moved to receptor fluid), to human split-thickness skin from 4 donors (dose volume = 200 µl/cm^2; application rate ≈ 2 mg/cm^2) in diffusion cell.	Following results relate to 0.1% in aqueous micellar solution and 0.3% oil-in-water emulsion, respectively: 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). Most of radioactivity in the skin remained in skin during additional 72-h period. Study results indicated that the value for the absorption of Polyaminopropyl Biguanide through the skin was 4.09%.[2]

Table 4. Dermal Penetration Studies

Ingredient	Animals/Protocol	Results
[^{14}C]- Polyaminopropyl Biguanide (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 %).[5]
[^{14}C]- Polyaminopropyl Biguanide (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 .[5]
20.2% aqueous Polyaminopropyl Biguanide (20.2% aqueous; specific activity = 1.4 MBq/mg)	Test substance warmed to 40°C and nominal concentrations up to 200 g/l applied (at volume of 10 µl/cm^2, unocluded and occluded) to human skin epidermal membranes in diffusion cell.	At a concentration of 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.[5]

Table 5. Toxicokinetic Studies

Ingredient	Animals/Protocol	Results
[^{14}C]- Polyaminopropyl Biguanide (20% aqueous in double deionized water; specific activity = 1.85 GBq/4 mmol)	Groups of Alpk:APfSD (Wistar-derived) rats (3 to 5/sex/group). Single oral dosage (20 mg/kg) administered by gavage. Labelled and unlabeled test substances fractionated into low, medium and high molecular weight (MW) fractions by centrifugation and also administered orally.	In bioavailability experiment (3 groups of 4 males), 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.[5]
[^{14}C]- Polyaminopropyl Biguanide (20% aqueous in double deionized water; specific activity = 1.85 GBq/4 mmol)	Groups of Alpk:APfSD (Wistar-derived) rats (5/sex/group) fed 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.[5]
Radiolabeled Polyaminopropyl Biguanide	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.[19]
Radiolabeled Polyaminopropyl Biguanide	Rats fed 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 Polyaminopropyl Biguanide oligomers with 2 cyanoguanidino end groups, as well as the trace constituents 3,3-dicyano-1,1-hexamethylenediguanidine and compound that was considered to be 1- (6-aminohexyl)-3-cyanoguanidine.[19]

Table 5. Toxicokinetic Studies

Ingredient	Animals/Protocol	Results
20% Polyaminopropyl Biguanide (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.[18]
20% Polyaminopropyl Biguanide	Groups of 3 male rats (strain not stated) maintained on diet that contained 100 ppm test substance	Concentration in abdominal fat peaked at 1.2 ppm after 3 weeks and was maintained at this level for another 2 weeks on diet. After returning to normal diet, concentrations in the abdominal fat reduced to 0.3 ppm after 5 weeks. Concentration in the liver did not exceed 0.6 ppm after 5 weeks of feeding, and was reduced to undetectable levels within 3 weeks of return to normal diet. Comparable concentrations (maximum) in the kidney and heart were 0.8 ppm and 0.1 ppm. Radioactivity not detected in brain.[18]
[^{14}C]- Polyaminopropyl Biguanide	10 NMRI mice received single oral dose of 2.0 mL by gavage and were then frozen in acetone at up to 48 h post-dosing. Whole body autoradiography subsequently performed (additional details not included).	No absorption detected[5]

Table 6. Acute Dermal Toxicity Studies

Ingredient	Animals	Protocol	Results
Polyaminopropyl Biguanide (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.[5]
Polyaminopropyl Biguanide (20% aqueous)	2 groups of 20 (10 males, 10 females/group) specific pathogen free (SPF) albino rats.	Topical application of test substance at doses of 2.5 ml/kg and 5 ml/kg, respectively. Test substance applied to intact skin and spread over area of ~1 $inch^2$. Site covered with patch for 24 h. 7-day observation period. Necropsy not performed.	No mortalities.[18]
Polyaminopropyl Biguanide (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).[5]

Table 7. Acute Oral Toxicity Studies

Ingredient	Animals	Protocol	Results
Polyaminopropyl Biguanide (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.[5]
25% aqueous Polyaminopropyl Biguanide	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.[18]
25% aqueous Polyaminopropyl Biguanide	3 female rats (strain not stated)	Single oral dose, followed by 7-day observation period.	No deaths and all organs appeared normal at necropsy.[18]
25% aqueous Polyaminopropyl Biguanide	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.[18]
20% aqueous Polyaminopropyl Biguanide	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.[5]
Polyaminopropyl 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% Polyaminopropyl 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.[20]

Table 8. Acute Inhalation Toxicity

Ingredient	Animals/Protocol	Results
Polyaminopropyl Biguanide (purity 99.6%) in aqueous solution	Wistar CRL:(WI) rats (groups of 10; 5/sex/test concentration. OECD Guideline 403-compliant study. Exposure levels (nose-only): 0.1, 0.3 and 0.5 mg/l 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.[5]
20.6% w/w Polyaminopropyl Biguanide	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.[5]
Polyaminopropyl Biguanide (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.[5] 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.[5]

Table 9. Short-Term Toxicity Studies

Ingredient	Animals	Protocol	Results
Dermal Studies			
25% aqueous Polyaminopropyl Biguanide	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.[18]
20.2% aqueous Polyaminopropyl Biguanide	Groups of 10 (5 males, 5 females per group) rats of the Alpk:APfSD (Wistar-derived) strain	Three groups received applications (occlusive, on back) 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.[5]
20% Polyaminopropyl Biguanide (diluted with water to 0.04% active ingredient)	5 female rats of Alderley Park strain.	0.04% applied (0.1 ml) to back on alternate days for total of 6 applications. No covering or test site covered with polyethylene secured with an adhesive plaster for 24 h.	No evidence of systemic toxicity (with or without covering).[18]
20% aqueous Polyaminopropyl Biguanide	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.[5]
Oral Studies			
25% aqueous Polyaminopropyl Biguanide	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.[18]
20% aqueous Polyaminopropyl Biguanide	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.[5]

Table 9. Short-Term Toxicity Studies

Ingredient	Animals	Protocol	Results
20% aqueous Polyaminopropyl Biguanide	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 effect). Treatment-related decrease in liver weight for males given 0.6 and 1.2 mg/ml, probably associated with poor nutritional status. Because effects on body weight and water consumption at all dose levels, NOAEL could not be derived. LOAEL = 0.1 mg/ml.[5]
Polyaminopropyl 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. No visible gross pathological changes in heart, liver and kidney samples. At 2 mg/kg and 8 mg/kg doses, mild toxicity in 50% of liver samples and 50% of kidney samples examined microscopically. At 32 mg/kg dose, mild toxicity in 50% of liver samples examined microscopically (mild kidney toxicity in 1 rat). In control group, mild toxicity (at microscopic examination) in kidneys of 30% of animals.[20]
Inhalation Studies			
19.2% aqueous Polyaminopropyl Biguanide	Groups of 10 (5 males, 5 females per group) rats of the Alpk:APfSD (Wistar-derived) strain	Three groups were exposed (nose-only) to concentrations of 0.025mg/m^3, 0.25 mg/m^3, and 2.5 mg/m^3, respectively, 6 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 Polyaminopropyl Biguanide 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.[5]

Table 9. Short-Term Toxicity Studies

Ingredient	Animals	Protocol	Results
20% aqueous Polyaminopropyl Biguanide	Groups of 8 (4 males, 4 females per group) SPF albino rats of the Alderley Park strain.	Five groups exposed (nose-only) to 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.[5]

Table 10. Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
		Oral Studies	
25% Polyaminopropyl Biguanide	Young adult SPF Wistar rats (25 males, 25 females/group)	90-day dietary study. Concentrations of 0 ppm, 2500 ppm, and 5000 ppm in diet.	No deaths during the 90-day feeding period. No gross abnormalities or abnormalities in hematological parameters. No remarkable changes in organ/body weight ratios. Microscopic examination revealed unusual degree of iron pigment in liver cells and in Kupffer cells for females fed 5000 ppm in the diet. Iron pigment not observed in liver of rats fed 2500 ppm in the diet (detailed histopathological results not included). Not possible to establish NOAEL.[18]
25% aqueous Polyaminopropyl Biguanide	Alderley Park Wistar Rats (number of animals not stated)	90-day dietary study. Concentrations of 0, 625 and 1250 ppm active ingredient	No mortalities. At 1250 ppm, deposits of an iron-pigment in liver (in hepatocytes and Kupffer cells) observed in female rats. No toxicity findings after feeding with 625 ppm.[5]
25% aqueous Polyaminopropyl Biguanide	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.[18]
25% aqueous Polyaminopropyl Biguanide	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.[5]
20.2% aqueous Polyaminopropyl Biguanide	Wistar -derived rats (Alpk:APfSD strain), 4 rats/sex/group	90-day dietary study. Concentrations: 0, 1000, 2000, 4000, and 6000 ppm active ingredient (corresponding to approximately 0, 83.9, 171.5, 373.0, 556.1 mg/kg 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).[5]

Table 10. Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
20.2% aqueous Polyaminopropyl Biguanide	C57Bl/10JfCD-1 mice, 4 mice/sex/group	90-day dietary study. Concentrations: 0, 1000, 2000, 4000 ppm active ingredient (corresponding to about 0, 162, 328, 736 mg/kg 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.[5]
20% aqueous Polyaminopropyl Biguanide	Mice of the C57BL/10JfAP/Alpk strain. 2 groups of 10 males and 10 females (1 test and 1 control)	90-day drinking water study. Test group dosed with 0.1 mg/ml during 1st week, 0.3 mg/ml during 2nd week, and 0.3 mg/ml from 3rd week until study termination.	Reduction in body weight gain and dose-related reduction in water consumption. No treatment-related macroscopic post-mortem findings.[5]

Table 11. Chronic Toxicity Studies

Ingredient	Animals	Protocol	Results
		Dermal Study	
Polyaminopropyl Biguanide	Four groups of SPF Alderley Park mice (50 males, 50 females/group)	Test substance (0.3 ml) administered daily at following doses 5 days per week for 80 weeks: 0 (in ethanol), 0.6 mg (0.2% test substance in ethanol), 6.0 mg (20% test substance) and 30 mg (10% test substance in ethanol).	High mortality rate (75% in males and females) in 30 mg/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. Tisues from other organs were also examined microscopically. [18]
		Oral Studies	
20.2% aqueous Polyaminopropyl Biguanide	Groups of 128 rats of the Alpk:APfSD (Wistar-derived) strain (64 males, 64 females per group)	Test substance administered in diet daily (for 104 weeks) at concentrations of 0 ppm, 200 ppm, 600 ppm, and 2000 ppm (corresponding to 0, ~12.1, ~36.3, and ~126.1 mg/kg 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. [5]

Table 11. Chronic Toxicity Studies

Ingredient	Animals	Protocol	Results
20.2% aqueous Polyaminopropyl Biguanide	Groups of 8 Beagle dogs (4 males, 4 females per group)	Test substance administered daily (for 1 year) at dietary concentrations of 0 ppm, 300 ppm, 1500 ppm, and 4500 ppm (corresponding to 0 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.[5]
20% Polyaminopropyl Biguanide	Groups of 30 male and 60 female SPF mice of the Alderley Park strain	Lifetime feeding study. 4 groups fed dietary concentrations of 0 ppm, 100 ppm, 200 ppm, and 1000 ppm, respectively, for 1 week prior to pairing and during mating. Feeding of females continued throughout pregnancy and lactation. All offspring were weaned at 3 weeks of age, and at 5 weeks of age, 50 males and 50 females were selected from each group. Offspring fed same diets as parents throughout study. Study terminated at 97 weeks after selection of the offspring, 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.[18]
20% Polyaminopropyl Biguanide	Four groups of SPF rats of the Alderley Park strain (60 males, 60 females per group)	122-week study. Dietary concentrations of 0 ppm, 200 ppm, 1000 ppm, and 2000 ppm, respectively. Study terminated at 124 weeks, 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.[18]

Table 11. Chronic Toxicity Studies

Ingredient	Animals	Protocol	Results
20% Polyaminopropyl Biguanide	Four groups of adult Beagle dogs (4 males, 4 females per group)	26-week study. Dietary concentrations of 0 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.[18]
Polyaminopropyl Biguanide	Strain not stated	Chronic toxicity study (protocol not stated).	NOEL = 200 mg/kg/day.[21]
Polyaminopropyl Biguanide	Strain not stated	2-year chronic toxicity study (protocol not stated). Dose: 100 mg/kg/day	No adverse effects.[21]

Table 12. Developmental and Reproductive Toxicity Studies

Ingredient	Animals	Protocol	Results
20.2% aqueous Polyaminopropyl Biguanide	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.[5]
20.2% aqueous Polyaminopropyl Biguanide	Groups of 20 female New Zealand White rabbits	Four groups received oral dosages (by gavage) of 0, 10, 20, and 40 mg/kg 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.[5]
20% aqueous Polyaminopropyl Biguanide	Groups of 30 Sprague-Dawley rats (10 males, 20 females per group).	Four groups received dietary concentrations of 0, 200, 650, and 1300 ppm (dietary levels adjusted for 20% active ingredient) during the 9-week 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.[5]
20% aqueous Polyaminopropyl Biguanide	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.[5]

Table 12. Developmental and Reproductive Toxicity Studies

Ingredient	Animals	Protocol	Results
20% aqueous Polyaminopropyl Biguanide (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.[2]
20% aqueous Polyaminopropyl Biguanide (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.[5]
20% aqueous Polyaminopropyl Biguanide	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.[5]

Table 12. Developmental and Reproductive Toxicity Studies

Ingredient	Animals	Protocol	Results
20% Polyaminopropyl Biguanide	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.[18]
0.04% Polyaminopropyl Biguanide	Animal strain not stated.	Oral dosing (test protocol not included)	Embryotoxic at dose of 32 mg/kg/day.[21]
Polyaminopropyl Biguanide	Rats (number and strain not stated)	Rats dosed orally with 100 mg/kg/day	Embryotoxic.[21]
Polyaminopropyl Biguanide	Rats (number and strain not stated)	Rats dosed intraperitoneally with 10 mg/kg/day	Teratogenic.[21]
20% aqueous Polyaminopropyl Biguanide	Groups of 8 (4 males, 4 females per group) SPF albino rats of the Alderley Park strain	In short-term toxicity study, 5 groups exposed (nose-only) to 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.[5]

Table 13. Genotoxicity Studies

Ingredient/Similar Chemical	Strain/cell type	Assay	Dose/Concentration	Results
In Vitro				
20% aqueous Polyaminopropyl Biguanide	*Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538	Ames test, with and without metabolic activation	333.3 mg (333,300 µg) per plate	Toxic at dose of 333.3 mg per plate, particularly in strains TA98, TA100, and TA1535. Weakly genotoxic in strain TA1538 without metabolic activation.[5]
20% aqueous Polyaminopropyl Biguanide	*Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538	Ames test, with and without metabolic activation	Doses up to 500µg/plate	Non-genotoxic.[5]
19.6% aqueous Polyaminopropyl Biguanide (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.[5]
20% aqueous Polyaminopropyl Biguanide	L5178Y TK+/- mouse lymphoma cells	Mouse lymphoma assay, with and without metabolic activation	Concentrations up to 100 µg/ml	At 50 and 100 µg/ml, cytotoxicity higher than that of positive controls. Non-genotoxic.[5]
20% aqueous Polyaminopropyl Biguanide	P388 (*tk*+/-) mouse lymphoma cell line	Mouse lymphoma assay, with and without metabolic activation	Concentrations up to 2000 µg/ml	2000 µg/ml was cytolethal and clear cytotoxicity noted at 1000 µg/ml, with and without metabolic activation. Non-genotoxic.[5]
19.6% aqueous Polyaminopropyl Biguanide	Cultured human peripheral blood lymphocytes from 2 volunteers	Micronucleus test	Concentrations up to 50 µg/ml without metabolic activation and concentrations up to 250 µg/ml with metabolic activation.	No chromosomal aberrations. Non-genotoxic.[5]
In Vivo				
19.6% aqueous Polyaminopropyl Biguanide	1000 polychromatic erythrocytes (from C57BL/6JfCD-1/Alpk mice) scored for presence of micronuclei	Micronucleus test.	Groups of 10 mice. Test substance administered (single dose, by gavage) at 0, 250, and 400 mg/kg body weight (dosage volume = 10 ml/kg body weight).	Non-clastogenic.[5]

Table 13. Genotoxicity Studies

Ingredient/Similar Chemical	Strain/cell type	Assay	Dose/Concentration	Results
19.6% aqueous Polyaminopropyl Biguanide	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.[5]

Table 14. Carcinogenicity Studies

Ingredient	Animals/Cells	Protocol	Results
		In Vitro Studies	
20% aqueous Polyaminopropyl Biguanide (in DMSO)	Baby hamster kidney fibroblasts (BHK21/C13)	Cell transformation assay, with metabolic activation. Test substances dose range of 0.25 - 2500 µg/ml and 25 -3000 µg/ml in separate experiments.	Cytotoxicity at 250 µg/ml and greater. No difference in number of transformed cell colonies between test and negative control cultures. Test substance did not induce cell transformation.[5]
Polyaminopropyl Biguanide (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.[2]
Polyaminopropyl Biguanide (up to 1 ppm)	RAW 264.7 mouse macrophages	Reactive oxygen species (ROS) assay. Macrophages cultured with Polyaminopropyl Biguanide (0, 0.75, and 1 ppm). Production of ROS in macrophages detected by measurement of fluorescence intensity after addition of dihydrorhodamine and by evaluation of tumor necrosis factor (TNF) α and interleukin (IL)-6 in cell culture medium, as quantified by the enzyme-linked immunosorbent assay (ELISA).	No activation of macrophages.[2]
		Dermal Studies	
Polyaminopropyl Biguanide (up to 20%)	Four groups of SPF mice (50 males, 50 females/group) of the Alderley Park strain	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% Polyaminopropyl Biguanide in ethanol), 6.0 mg (20% Polyaminopropyl Biguanide) and 30 mg (10% Polyaminopropyl Biguanide 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 as hepatic oncogene in mice

Table 14. Carcinogenicity Studies

Ingredient	Animals/Cells	Protocol	Results
			dosed with 30 mg.[18]
20% aqueous Polyaminopropyl Biguanide	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.[5]
Oral Studies			
20.2% aqueous Polyaminopropyl Biguanide	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.[5]
20.2% aqueous Polyaminopropyl Biguanide	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. According to the SCCS, these data considered to be of low reliability due to high mortality.[5]

Table 14. Carcinogenicity Studies

Ingredient	Animals/Cells	Protocol	Results
20.2% aqueous Polyaminopropyl Biguanide	Groups of 30 male and 60 female SPF mice of the Alderley Park 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.[18]
20.2% aqueous Polyaminopropyl Biguanide	Groups of 60 male and 60 female rats of unspecified strain	4 groups fed at concentrations of 0, 200, 1000 and 2000 ppm	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.[5]
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.[18]
20% Polyaminopropyl Biguanide	SPF rats (60 males, 60 females per group) of the Alderley Park strain	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.[18]

Table 14. Carcinogenicity Studies

Ingredient	Animals/Cells	Protocol	Results
Polyaminopropyl Biguanide	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).[2]
Polyaminopropyl Biguanide	Groups of Wistar-derived Alpk:ApfSD rats	Concentrations of 0, 200, 600 or 2000 ppm (approximately equivalent to 0, 12.1, 36.3 and 126.1 mg/kg/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.[22]

Table 15. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
		Irritation Studies	
Animal Studies			
Polyaminopropyl Biguanide	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.[5]
Polyaminopropyl Biguanide (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.[2]
25% aqueous Polyaminopropyl Biguanide	3 female rats (strain not stated)	Test substance applied (dose per cm^2 or dose volume 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 2^{nd} and 3^{rd} applications, by which time there was pronounced edema.[18]
25% aqueous Polyaminopropyl Biguanide	2 groups of 20 (10 males, 10 females/group) healthy SPF albino rats	2 groups received a topical application of test substance to intact skin at 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.[18]
25% aqueous Polyaminopropyl Biguanide	Albino guinea pigs (6 test and 4 control) of Porton strain	Both ears treated (patch application; 0.1 ml per ear) with 25% 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%.[5]
20.2% aqueous Polyaminopropyl Biguanide	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.[5]

Table 15. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
20% aqueous Polyaminopropyl Biguanide	9 (3 males, 6 females) New Zealand White rabbits	Test substance applied to 6 rabbits (0.5 ml, under occlusive dressing) for 24 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.[5]
20% aqueous Polyaminopropyl Biguanide	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.[5]
20% aqueous Polyaminopropyl Biguanide	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.[5]
20% aqueous Polyaminopropyl Biguanide	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.[18]
20% aqueous Polyaminopropyl Biguanide	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.[5]
Polyaminopropyl Biguanide (0.2% in ethanol, 10% in ethanol and 20% [solvent not stated])	4 groups of SPF Alderley Park mice (50 males, 50 females)	Test substance (0.3 ml) was administered at the following doses 5 days per week for 80 weeks: 0 (in ethanol), 0.6 mg (0.2% Polyaminopropyl Biguanide in ethanol), 6.0 mg (20% Polyaminopropyl Biguanide) and 30 mg (10% Polyaminopropyl Biguanide in ethanol).	The 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.[18]

Table 15. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
Human Studies			
20% aqueous Polyaminopropyl Biguanide	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.[5]
Bacterial nanocellulose dressing loaded with 1% w/v sericin and 0.3% w/v Polyaminopropyl 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.[26]
Sensitization Studies			
In Vitro Assay			
Polyaminopropyl Biguanide		Local lymph node assay (protocol details not stated)	Positive results.[31]
Animal Studies			
20.2% aqueous Polyaminopropyl Biguanide	20 female Alpk:Dunkin Hartley guinea pigs (test group) and 10 female guinea pigs (control group)	Guinea pig maximization test. Induction phase: intradermal induction (0.3 % of test substance as delivered [0.06 % active ingredient], 0.1 ml in shoulder region). One week later, dermal induction performed by occlusively applying neat substance (20.2 % active ingredient) to induction sites for 48 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.[5]
20.2% Polyaminopropyl Biguanide (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.[5]
20% aqueous Polyaminopropyl Biguanide	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.[5]

Table 15. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
20% aqueous Polyaminopropyl Biguanide	Female Dunkin Hartley guinea pigs (20 test and 8 control animals).	Guinea pig maximization test. Possible cross-reactivity with chlorhexidine also evaluated. Intradermal induction with 0.25% 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.[5]
20% aqueous Polyaminopropyl Biguanide	10 Alderley Park guinea pigs (test animals) and 10 control guinea pigs.	Buehler test. Concentration of 10% (2% active ingredient, 0.4 ml) applied to scapular region 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 faint erythema at concentrations of 4% (8 of 9 animals) and 2% (3 of 10 animals) active ingredient. 2% active ingredient considered moderate sensitizer.[5]
20% aqueous Polyaminopropyl Biguanide	Groups of 20 (10 males and 10 females per group) guinea pigs	Buehler test. Induction and challenge concentrations: induction (0.3%) and challenge (0.3%, 0.15%, 0.075%, and 0.03%); induction (0.8%) and challenge (0.8%, 0.4%, 0.2%, and 0.08%); induction (1.3%) and challenge (1.3%, 0.65%, 0.325%, and 0.13%); induction (1.8%) and challenge (1.8%, 0.9%, 0.45%, and 0.18%); induction (2%), challenge (2%), and rechallenge (2%); 1.2% induction, challenge (1.2%), and rechallenge (1.2% and 15%); and induction (5%), challenge (15%), and rechallenge (2% and 1.2%).	Threshold for eliciting sensitization in guinea pigs was approximately 1%.[5]

Table 15. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
Human Studies			
20% aqueous Polyaminopropyl Biguanide	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.[5]
20% aqueous Polyaminopropyl Biguanide	1554 male and female patients	Multicenter study. Patch tests (performed in accordance with recommendations of the International Contact Dermatitis Research Group [ICDRG] and the German Contact Dermatitis Research Group [DKG]) on 2.5% aqueous test substance (effective concentration = 2.5% 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.[29]
20% aqueous Polyaminopropyl Biguanide	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.[32]
2.5% aqueous Polyaminopropyl Biguanide	374 patients (multicenter study in United Kingdom)	Patch test (protocol not stated)	2 positive patch test reactions. Data series suggested that baseline frequency of Polyaminopropyl Biguanide sensitization was very low (0.5%) in United Kingdom. Majority of reactions were weak, and data suggested that Polyaminopropyl Biguanide may not be a relevant contact allergen.[28]

Table 15. Dermal Irritation and Sensitization Studies

Ingredient	Number of Animals/Subjects	Protocol	Results
2.5% aqueous Polyaminopropyl Biguanide	1154 patients (multicenter study in Germany)	Patch test (protocol not stated)	6 positive patch test reactions. Data series suggested that baseline frequency of Polyaminopropyl Biguanide sensitization was very low (0.4%) in Germany. Majority of reactions were weak, and data suggested that Polyaminopropyl Biguanide may not be a relevant contact alldrgen.[29]
2.5% aqueous Polyaminopropyl Biguanide	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.[30]
Phototoxicity/Photosensitization Studies			
Animal Study			
20% aqueous Polyaminopropyl Biguanide	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.[18]
Human Study			
20% aqueous Polyaminopropyl Biguanide	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.[18]

Table 16. Ocular Irritation Studies

Ingredient	Number of Animals	Test Protocol	Results
Polyaminopropyl Biguanide (powder form, 99.6% pure)	1 New Zealand rabbit	Test substance (0.1 g) instilled into 1 eye.	Moderate redness, chemosis, moderate corneal opacity, iridial congestion, and ulceration of the nictitating membrane and cornea. Severe ocular irritant.[5]
Polyaminopropyl Biguanide (undiluted)	1 male New Zealand White rabbit	Test substance (0.1 ml) instilled into conjunctival sac of right eye; untreated eye served as control. Eye not rinsed after instillation.	Opalescent corneal opacity, iridial inflammation, and severe conjunctival irritation observed initially. Translucent corneal opacity, minimal conjunctival irritation and vascularization were noted at day 21 post-instillation and considered irreversible reactions. Test substance was corrosive to rabbit eye.[5]
25% aqueous Polyaminopropyl Biguanide	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.[18]
20% aqueous Polyaminopropyl Biguanide	9 female New Zealand White rabbits	Test substance (0.1 ml) instilled into conjunctival sac of 1 eye; contralateral eye served as untreated control. Eyes of 6 animals not rinsed after instillation. Eyes of remaining 3 animals were rinsed.	Iritis and conjunctivitis in unrinsed eyes and 4/6 rabbits with transient corneal opacity. Conjunctivitis, but no corneal reaction, in rinsed eyes and slight iritis in 1 rabbit. Test substance was moderate eye irritant in unrinsed eyes and a mild irritant in rinsed eyes.[5]
20% Polyaminopropyl Biguanide	3 rabbits (strain not stated)	Test substance (0.12 ml) instilled into 1 eye, followed by rinsing with saline	Slight inflammation, but no corneal ulceration. Changes resolved in 10 days.[18]
20% Polyaminopropyl Biguanide	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.[18]
20% aqueous Polyaminopropyl Biguanide	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.[33]

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2017 FDA VCRP Data

Polyaminopropyl Biguanide

03C - Eye Shadow	2
03D - Eye Lotion	3
03E - Eye Makeup Remover	8
03F - Mascara	12
03G - Other Eye Makeup Preparations	3
04E - Other Fragrance Preparation	1
05A - Hair Conditioner	5
05F - Shampoos (non-coloring)	1
05G - Tonics, Dressings, and Other Hair Grooming Aids	7
05H - Wave Sets	1
05I - Other Hair Preparations	2
07C - Foundations	4
07E - Lipstick	1
07H - Makeup Fixatives	1
07I - Other Makeup Preparations	6
08C - Nail Creams and Lotions	1
08G - Other Manicuring Preparations	1
10A - Bath Soaps and Detergents	2
10E - Other Personal Cleanliness Products	7
11A - Aftershave Lotion	1
12A - Cleansing	20
12C - Face and Neck (exc shave)	18
12D - Body and Hand (exc shave)	13
12F - Moisturizing	12
12G - Night	6
12H - Paste Masks (mud packs)	1
12I - Skin Fresheners	4
12J - Other Skin Care Preps	4
Total	**147**

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: October 31, 2016

SUBJECT: Concentration of Use by FDA Product Category: Polyaminopropyl Biguanide

Concentration of Use by FDA Product Category - Polyaminopropyl Biguanide

Product Category	Maximum Concentration of Use
Baby lotions, oils and creams 1B	0.1%
Eye shadows 3C	0.03%
Eye lotions 3D	0.04-0.2%
Eye makeup removers 3E	0.04-0.28%
Mascara 3F	0.1-0.3%
Other eye makeup preparations 3G	0.01%
Hair conditioners 5A	0.00025-0.3%
Hair sprays 5B	
Aerosol	0.00025-0.0004%
Pump spray	0.000002-0.27%
Hair straighteners 5C	0.01%
Shampoos (noncoloring) 5F	0.008%
Tonics, dressings and other hair grooming aids 5G	0.000023-0.5%
Other hair preparations (noncoloring) 5I	0.002%
Hair dyes and colors 6A	0.5%
Foundations 7C	0.01%
Deodorants 10B	
Not spray	0.003%
Other personal cleanliness products 10E	0.006%
Skin cleansing (cold creams, cleansing lotions, liquids and pads) 12A	0.02-0.5%
Face and neck products 12C	
Not spray	0.01-0.24%
Body and hand products 12D	
Not spray	0.00001-0.009%
Moisturizing products 12F	
Not spray	0.00075%
Skin fresheners 12I	0.43%
Suntan products 13A	
Not spray	0.002-0.1%

Information collected in 2016

Table prepared October 28, 2016

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: February 21, 2017

SUBJECT: Polyaminopropyl Biguanide

Anonymous. 2017. Supplier comments on the identity of Polyaminopropyl Biguanide.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

February 2017

Supplier Comments on the Identity of Polyaminopropyl Biguanide

Effectively all PHMB is Poly (hexamethylene Biguanide) HCl. That is C6 alkyl chains linked by Biguanide groups.

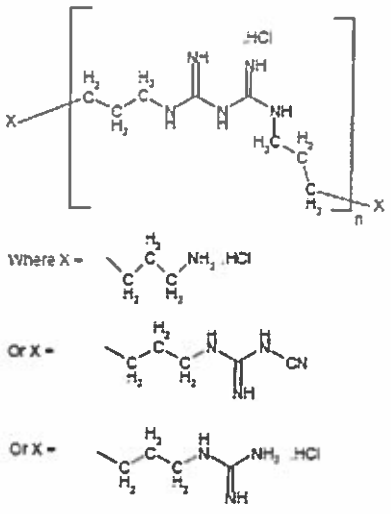
There are no propyl Biguanide groups present (this is an artifact of the INCI name as the polymer repeating unit has been arbitrarily chosen as the middle of the C6 alkyl chain).

Chemical names:-
IUPAC Name: Homopolymer of N-(3-Aminopropyl)-Imidodicarbonimidic Diamide

Poly(hexamethylenebiguanide hydrochloride)
Poly(iminocarbonimidoyliminocarbonimidoylimino-1,6-hexanediyl), hydrochloride
Poly(iminoimidocarbonyl-iminoimidocarbonyl-iminohexamethylene), hydrochloride
Poly(iminoimidocarbonyliminoimidocarbonyliminohexamethylene) hydrochlorid

The structure is given in the SCCS Opinion
https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_204.pdf

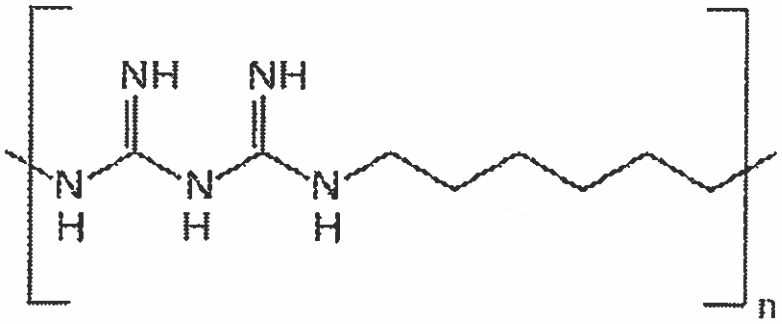
Structural Formula:



Where n = 1 to 40 and average molecular weight corresponds to n = 10 - 13

From a chemical perspective this is better represented as

$-(CH_2\text{-}CH_2\text{-}CH_2\text{-}\ CH_2\text{-}CH_2\text{-}CH_2\text{-NH-CNH-NH-CNH-NH-}) - CH_2\text{-}CH_2\text{-}CH_2\text{-}\ CH_2\text{-}CH_2\text{-}CH_2$



Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: February 21, 2017

SUBJECT: Polyaminopropyl Biguanide

Cosmetics Europe. 2016. Dossier on the Safety of Poly(hexamethylene) biguanide hydrochloride or Polyaminopropyl Biguanide (PHMB) (CAS Nos. 32289-58-0 or 27083-27-8) in Cosmetic Products Submission III.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Dossier

on the Safety of

Poly(hexamethylene) biguanide hydrochloride

or

Polyaminopropyl Biguanide

(PHMB)

(CAS Nos. 32289-58-0 or 27083-27-8)

in Cosmetic Products

Submission III

Sponsor: Cosmetics Europe
Avenue Herrmann-Debroux 40
B-1160 Brussels, Belgium

Date: 24 May 2016

Submission I **January 2014**
Submission II **September 2014**

Table of contents

Background:

This Submission III Safety Dossier is a stand-alone submission containing all the datasets submitted to the SCCS so far, i.e. Submission I (January 2014) and Submission II (September 2014) as well as additional datasets as outlined in the present Submission. In response to the SCCS request for additional dermal absorption studies on representative cosmetic formulations containing PHMB (SCCS, Ref 100, 2015), new *in vitro* skin penetration data have been generated with two representative cosmetic formulations. Thus, two new percutaneous absorption studies using human skin were included in section 6.4.1.

Currently, PHMB is authorized as a preservative in cosmetic products up to a maximum concentration of 0.3% as listed in Annex V. However, taking account of the last SCCS Opinion on PHMB (SCCS, Ref 100, 2015), a cosmetic industry-wide evaluation of PHMB usage and based on the outcome of the recently completed studies, Industry considers it can support a maximum concentration of 0.1% PHMB in cosmetic products in order to safeguard an appropriate MoS and to alleviate the concerns raised by the SCCS in their aforementioned opinion. Information from Industry and from the market shows that this concentration is effective and typically used within cosmetic products.

The hazard assessment (hazard identification and dose-response assessment) presented in this Submission III safety dossier is in accordance with the recent SCCS opinion (SCCS, Ref 100, 2015) including the No-Adverse-Observed-Effect Level (NOAEL) of 36 mg/kg bw/day which was taken as Point of Departure (PoD) for the safety evaluation and MoS calculation.

1. Executive summary

The toxicity profile of Poly(hexamethylene) biguanide hydrochloride or Polyaminopropyl Biguanide (PHMB), a preservative used in cosmetic products, was extensively investigated in pre-clinical studies assessing acute and repeated dose toxicity, irritation, skin sensitisation, percutaneous absorption, genotoxicity, carcinogenicity, reproductive toxicity, prenatal developmental toxicity, toxicokinetics, and mechanistic studies on the carcinogenic potential.

Content redacted as this is EU specific

1A Summary of Toxicity Data on PHMB

Acute toxicity

In accordance with the recent SCCS opinion (SCCS, Ref 100, 2015), the acute toxicity of PHMB can be considered as moderate to low after oral and dermal administration, respectively. PHMB is considered toxic after inhalation. However, there is evidence from the clinical signs observed, that the acute inhalation toxicity of PHMB is caused more by local than by systemic effects.

Irritation and corrosivity

In accordance with the recent SCCS opinion (SCCS, Ref 100, 2015), PHMB can be considered as irritating to skin. Whereas neat PHMB is corrosive to the eye, 20% solutions of PHMB can be considered as moderately irritating to the eye. PHMB can also be considered irritating to the respiratory tract leading to 50 % reduction of respiratory rate at 264 mg/m^3.

Skin sensitisation

In accordance with the recent SCCS opinion (SCCS, Ref 100, 2015), PHMB can be considered as a moderate to strong sensitizer in guinea pigs based on various maximization- and Buehler tests. The threshold for eliciting sensitisations in guinea pigs is approximately 1 %. From HRIPT tests it can be concluded that a concentration of 2% PHMB is capable of causing skin sensitisation humans which can be elicited at concentrations starting from 0.2 % a.i. From reports on patch tests, PHMB sensitization in humans can be considered of low frequency (up to 0.5 % in dermatitis patients). At a use concentration of 0.1% PHMB in cosmetic products, there is a negligible risk for the consumer with respect to skin sensitization.

Repeated dose toxicity

Repeated dose toxicity studies using different durations (3 weeks up to 2 years) and different application routes (oral – drinking water; oral – diet; dermal; inhalation) have been performed in different animal species (rats, mice, dogs). Short-term and subchronic oral studies mainly served as range finder for studies of longer duration. Subchronic dietary studies performed in rats and mice caused reductions in body weight and effects on weight gain. In rats, the kidney was identified as target organ; there were changes in renal function as well as changes in some plasma parameters. However, interpretation of the dietary subchronic studies was hampered by limited histopathologic examination.

The subacute occlusive dermal administration of PHMB led to no sign of systemic toxicity in male and female rats up to the highest dose level of 200 mg/kg bw/day. Signs of dermal irritation were noted but a clear NOAEL of 20 mg/kg bw/day for this local effect was achieved.

In a dietary 12-month study in beagle dogs, a NOAEL of 54 mg/kg bw was derived. At the highest dose tested there were adverse effects on scrotal skin, indications of liver impairment and histopathological findings in skin, liver and – for males only – in kidneys and testes.

In a dietary 2-year rat study, a NOAEL of 600 ppm was derived (equivalent to about 36 and 45 mg/kg bw/d in male and female animals, respectively). At the highest dose tested, most prominent findings were reductions in body weight, changes in plasma parameters, the incidence of hepatocyte fat and spongiosis hepatis. Further, the incidence of haemangiosarcomas was increased at the highest dose tested. In accordance with the recent SCCS opinion (SCCS, Ref 100, 2015), the NOAEL of 36 mg/kg bw/day in males will be used as the most representative lowest value for repeated dose toxicity for the calculation of the Margin of Safety (MoS).

Mutagenicity

In accordance with the recent SCCS opinion (SCCS, Ref 100, 2015), PHMB can be considered to have no genotoxic potential based on the available data.

The bacterial reverse mutation test is considered not suitable to be used as PHMB is a bactericide. PHMB was negative in a gene mutation assay in mammalian cells and in an *in vitro*

chromosome aberration test in human lymphocytes. An *in vitro* micronucleus test was also negative but only short treatment times were used. The negative results found in *in vitro* tests were confirmed under *in vivo* conditions as PHMB did not lead to the induction of micronucleated polychromatic erythrocytes in mice. These negative results in the *in vivo* micronucleus test were further supported by a negative *in vivo* UDS test.

Carcinogenicity

In a rat oral chronic toxicity/carcinogenicity assay, a statistically not significant increase in the incidence of haemangiosarcomas was observed at the highest dose of 2000 ppm PHMB. Recently it has been concluded by ECHA's RAC committee that evidence from the chronic rat study is not sufficient to demonstrate a clear treatment-related effect. In an oral study in mice, PHMB increased the incidence of vascular tumors, mainly in the liver. In a dermal study in mice, a statistically significant increase in the incidence of haemangiosarcomas was observed in females at the highest dose tested (750 mg/kg bw/d; considered to exceed MTD). Despite the weak evidence of the carcinogenic potential of PHMB, RAC recently concluded that classification as Carc 2 H351 (suspected of causing cancer) according to CLP would be appropriate.

Reproductive toxicity

In an oral chronic study in Beagle dogs, histopathologic changes in testes were observed at the highest dose tested (169 mg/kg bw/d, reduced to 108 mg/kg bw/d). In a 2- and a 3-generation reproductive toxicity study in rats, no adverse effects on the reproductive parameters addressed were observed. No evidence of foetal/developmental toxicity independent from maternal toxicity was observed in developmental toxicity studies in rats and rabbits. There were indications for retarded ossification at 20 and 40 mg/kg bw/d in a developmental toxicity study in mice, which was not considered an adverse effect by the SCCS.

Toxicokinetics

Screening studies on oral absorption, distribution, metabolism and excretion in rats with non-labelled PHMB as 20% aqueous solution and [14C]-labelled PHMB fractionated into low, medium and high molecular fractions indicated that PHMB is poorly absorbed by the oral route. The vast majority of PHMB remained unabsorbed and was excreted mainly via the faeces.

From an oral (gavage) single dose toxicokinetic study using different molecular weight fractions of PHMB, up to 8.5 % of the applied radioactivity might be considered bioavailable (sum of urinary excretion and radioactivity in tissues and residual carcass at study termination) which might be rounded to 10 %.

Thus, in line with the recent SCCS opinion (SCCS, Ref 100, 2015), a value of 8.5% for oral absorption was used to correct the most representative lowest NOAEL for repeated dose toxicity for the calculation of the Margin of Safety (MoS).

Dermal absorption

In Submission I, the available *in vitro* studies on percutaneous penetration of PHMB were not deemed in compliance with SCCS requirements, qualities of the studies were considered to be compromised by poor sample description and the studies have not been performed with

representative cosmetic formulations containing PHMB. Therefore for Submission II an additional dermal penetration study was performed with radiolabelled PHMB at 0.3% in a representative cosmetic formulation (oil in water emulsion, o/w) according to OECD TG 428 and SCCS basic requirements for dermal penetration studies and in line with GLP principles (Ref 108, 2014). A total of 20 tape strippings was performed and the PHMB present in each tape stripping was quantified to produce a profile of the absorbed PHMB through the stratum corneum/upper epidermis. The SCCS considered the study acceptable, but decided to use the mean of the dermally delivered dose plus 2 SD, i.e. 3.49 + 4.16 % = 7.65 % of the applied dose, as skin absorption rate for the calculation of the Systemic Exposure Dose (SED). This decision was particularly based on the number of tape strippings used to remove stratum corneum which was deemed unusually high (n=20), the fact that dermal penetration was only investigated in one type of cosmetic formulation (o/w emulsion), and the variability observed in the study.

In order to respond to the key comments of the SCCS, and also to evaluate whether the amounts of PHMB found in the stratum corneum and epidermis are available for systemic exposure, two new *in vitro* dermal penetration studies were performed, one with 0.3% PHMB (current maximum concentration permitted according to Annex V of the Cosmetics Regulation, previously tested concentration) and one with 0.1% PHMB. Both were conducted with two different representative cosmetic formulations, one which facilitates penetration (o/w emulsion) and one which is the most representative of products on the market (aqueous micellar solution), using a methodology consistent with both the OECD and the SCCS requirements (Ref 109, 110, 2016ab).

The objective of the first new study was to determine whether the amount of PHMB found in skin compartments of the previous 24 h standard study with 0.3% PHMB in o/w emulsion represented a genuine skin reservoir for potential systemic exposure or whether these residues are systemically unavailable and, therefore, can be reasonably excluded. Thus, the study design was based on a refined test protocol including a standard 24 h exposure to 0.3% PHMB in two different representative cosmetic formulations (aqueous micellar solution, o/w emulsion), measurement of penetration into receptor fluid at 24 h up to 72 h and determination of PHMB remaining in the stratum corneum, epidermis, and dermis at 72 h.

The second new study assessed the *in vitro* skin penetration of 0.1% PHMB in two different representative cosmetic formulations (aqueous micellar solution, o/w emulsion) according to the SCCS and OECD guidelines with the usual exposure and monitoring time of 24 h in order to provide the skin penetration rate for the proposed new lower limit of 0.1% for PHMB. No measurement was performed at 72 h as evidence was obtained in the previous study at 0.3% that there was no reservoir effect.

The table below illustrates the design of the new *in vitro* dermal penetration studies and compares it with the previous study:

Submission	PHMB concentration	Exposure time	Monitoring time	Tape stripping	Cosmetic formulations
II	1. 0.3%	2. 24 h	3. 24 h	4. 20	5. o/w emulsion
6. III	7. 0.3%	8. 24 h	9. 24 h & 72 h	10. 5	11. aqueous micellar solution

					o/w emulsion
12. III	13. 0.1%	14. 24 h	15. 24 h	16. 5	17. aqueous micellar solution o/w emulsion

According to the SCCS, it cannot be excluded that some absorbable amounts of PHMB were removed by the high number of tape strippings used. Thus, the number of tape strippings was reduced to 5 in both new studies; PHMB is a highly substantive polymer which binds strongly to the stratum corneum. The amount of residual stratum corneum was higher after only 5 tape strippings leading to a corresponding higher level of PHMB absorbed in the "residual stratum corneum + epidermis" layer compared to the previous skin penetration study at 0.3% PHMB with 20 tape strippings.

In general, a number of 15 to 25 tape strippings may be used in the *in vitro* skin penetration assay (OECD, 2004) which still leaves some residual stratum corneum present. It is therefore not realistic to assume that skin rinsing followed by 5 tape strippings would remove all residues of the applied substance from the outer skin surface. Tape stripping experiments *in vitro* on excised human skin are confounded by the loss of the flexibility and elasticity of the skin. As a result, corneocytes (biologically dead stratum corneum cells) remain in hair follicle openings, skin furrows and wrinkles after the tape stripping procedure (Ref 74, 75, 2005 and 2008). However, most importantly both studies demonstrated that although the amount of PHMB absorbed onto the residual stratum corneum was higher, it did not penetrate further to produce systemic exposure.

Overall, mass balance of the new studies was 91-99%. At least 50% of the applied PHMB did not enter the skin at all, similar to the previous skin penetration study at 0.3% PHMB. The results on both test formulations clearly showed that the total amounts of PHMB penetrating the skin (receptor fluid plus receptor chamber wash) were negligible, i.e. with a mean below the limit of reliable measurement of 30 dpm above background. Both with 0.3% and 0.1% PHMB formulations, the majority of the supposedly absorbed dose (85-95%) was detected in the "residual stratum corneum + epidermis" layer, i.e., which points to the fact that PHMB does not penetrate further. The kinetics of skin penetration over the 72-h monitoring period showed that the PHMB content in the receptor fluid did not increase from 24 to 72 h. This clearly demonstrated that PHMB present in the "epidermis + lower layers of stratum corneum" reflects absorption to the residual stratum corneum only and that these PHMB fractions do not act as a reservoir for continued percutaneous penetration and subsequent systemic bioavilability. On the basis of these data the PHMB amounts present in the "epidermis + lower layers of stratum corneum" can be reasonably excluded from the calculation of the SED. Thus, the SED is considered to consist solely of the amounts of PHMB present in the receptor fluid and in the dermis.

In summary, this approach is scientifically justified and acceptable for the following reasons:

- The newly generated dermal penetration data with 0.3% PHMB in two representative cosmetic formulations and an extended monitoring time up to 72 h clearly demonstrate that the PHMB fractions present in the "residual stratum corneum + epidermis" do not act as a reservoir for continued percutaneous penetration and subsequent systemic bioavilability.

- The 9th Revision of the Notes of Guidance (SCCS, Ref 101, 2016) states that "*in the case of substances with very low dermal absorption and limited permeation, the epidermis may be excluded when it is demonstrated that no movement of the chemicals from the skin reservoir to the receptor fluid occurs"*
- The physicochemical properties of the polycationic polymer PHMB are indicative of low or very low absorption, i.e. high MW of >700 Da with average molecular weights in the range between 2670 and 4216 Da, high degree of ionisation, low log P_{ow} of -2.3 at 25°C, pH 7.4 (SCCS, Ref 100, 2015). This position is supported by a retrospective study of those substances listed in the Cosmetic Regulation Annexes and present in the Opinions (2000-2014) of the SCCS (SCCS, Ref 101, 2016).
- The same approach was applied and accepted in a previous SCCS Opinion on hair dye reaction products (SCCS, Ref 99, 2010).

The interpretation of the experimental results from the new *in vitro* skin penetration studies was strengthened by an independent Expert Opinion (Ref 49, 2016), outlining that the new data confirm that PHMB is a very poor skin penetrant and that its transfer from a topical product across the skin and into the systemic circulation is extremely limited. A substantial fraction of PHMB that nominally resides in the "residual stratum corneum + epidermis" is located in the residual stratum corneum, i.e. in the non-living part of the skin, and therefore unavailable for potential dermal penetration. This is strongly supported by the fact that the percentage of radioactivity in this compartment did not change significantly when the monitoring time was increased from 24 to 72 h. This means, the compound present in this tissue was not mobile and did not migrate further into the skin. According to the independent expert, it seems very unlikely that any radiolabelled PHMB recovered from the „stratum corneum + epidermis" layer would ever contribute significantly to the body burden (SED) of PHMB.

As indicated above, already the physicochemical properties of the polycationic polymer PHMB as a whole are indicative of low or very low skin penetrability. Moreover, a positively charged polymer like PHMB has a strong affinity to the stratum corneum and will therefore bind to the negatively charged skin surface. This view has recently been confirmed by the Danish EPA in their Assessment of Nano-enabled Technologies in Cosmetics, stating that "*surface charge is a major contributor to avoid dermal delivery to systemic circulation due to the interaction of positively charged particles with the negatively charged skin cells" (*Ref 15, 2013; Ref 96, 2016).

Overall, in order to estimate the SED of PHMB on the basis of *in vitro* skin penetration datasets, it is reasonable to use the total amounts of PHMB penetrating the skin (receptor fluid plus receptor chamber wash) plus the amounts found in the dermis but to exclude the amounts found in the "epidermis + lower layers of stratum corneum". Given the physicochemical properties of the polycationic polymer PHMB, this approach is considered sufficiently conservative.

For the two cosmetic formulations with the reduced maximum use concentration of 0.1% for PHMB, the mean skin penetration rates were quite similar, *i.e.* 1.58 and 1.06%. Since this study was fully compliant with the requested guidelines and incorporated more than one type of cosmetic formulations, only 1 SD was added as required by the SCCS Notes of Guidance, resulting in skin penetration rates of **4.09%** (1.56% in dermis + 0.03% total absorbed + 2.5% *i.e.* 1 SD) in aqueous micellar solution and **1.94%** (1.02% in dermis + 0.04% total absorbed + 0.88% *i.e.* 1 SD) in o/w emulsion.

The difference to the skin penetration rate of 7.65% from the previous submission is due to the reduced maximum use concentration of 0.1% for PHMB (*vs* 0.3%), the use of 1 SD (*vs* 2 SD), the use of 5 tape strippings (*vs* 20 tape strippings) in order to remove the stratum corneum, and the exclusion of the amounts found in the epidermis and remainders of the stratum corneum.

1B Aggregate Exposure Assessment

Assessment of Exposure from Cosmetics

Exposure assessments should include an iterative process, and should be conducted using a tiered strategy. Starting with a semi-quantitative estimate such as a deterministic estimate with conservative assumptions, this approach can be refined with a more realistic estimation of population exposure that is modelled using probabilistic methods and a person-oriented approach.

Content redacted as this is EU specific

Using the **deterministic exposure assessment** as outlined in the SCCS Notes of Guidance, an aggregate exposure value of 17.4 g/d to cosmetic products is to be used for preservatives. This value is based on the traditional approach to modelling exposure assuming that all cosmetic products contain the same preservative at the maximum use concentration and are all are used by all persons at a high amount per use, and at a high frequency per day. Moreover, the aggregate exposure is based on the summation of all individual product exposures thus leading to an ‘unrealistic scenario’ type calculation.

1C Margin of Safety Calculations

In the most reliable chronic toxicity study in rats, the oral (diet) administration of PHMB for a period of up to two years led to reduced survival (females), reduced body weight (both sexes) and changes indicative for a slight liver impairment. The NOAEL was 600 ppm (equivalent to about **36 mg/kg bw/day** in males) and will be used as the most representative lowest value for repeated dose toxicity for the calculation of the Margin of Safety (MoS) in accordance with the recent SCCS opinion (SCCS, Ref 100, 2015).

Content redacted as this is EU specific

As mentioned above, on the basis of the newly generated dermal penetration data and supported by the 9th Revision of the Notes of Guidance as well as by an independent expert review of the available experimental and physicochemical data, the “residual stratum corneum + epidermis” fractions were not considered as contributing to the SED. For the MoS calculation, the skin penetration rate of the aqueous micellar solution containing 0.1% PHMB was taken since it is the representative formulation for a large range of cosmetic products using PHMB. Moreover, the skin penetration rate is higher than with the o/w emulsion formulation and thus, represents a more conservative approach.

Following a **deterministic exposure assessment** for general use of PHMB in cosmetic products, aggregate exposure amounts to **17.4 g/d** based on the product use data listed in Table 4 of the SCCS Notes of Guidance 9th Revision (SCCS, Ref 101, 2016). This assessment conservatively assumes that consumers will be exposed daily, throughout their life, to each of the 17 product types, each of them containing PHMB at the maximum concentration of use.

1.1 Summary of reliable toxicity data

Acute toxicity

The acute oral LD_{50} of PHMB was 1049 mg/kg bw in female Sprague-Dawley rats.

The acute dermal LD_{50} of PHMB was >5000 mg/kg bw for male and female Sprague-Dawley rats.

The acute inhalation LC_{50} of PHMB was 0.29 mg/L/4 h for males, 0.48 mg/ L/4 h for females or 0.37 mg/ L/4 h for males and females combined.

Skin and mucous membrane irritation

The application of neat PHMB to the skin of rabbits led to no signs of skin irritation. Dermal application of a 20% aqueous PHMB solution caused moderate but completely reversible erythema.

Neat PHMB led to irreversible ocular damage and was considered as corrosive to the rabbit eye.

Skin sensitization in experimental animals

PHMB was shown to be a skin sensitizer in Guinea pigs in Maximisation and Buehler tests. The threshold concentration of PHMB for induction of sensitization in guinea pigs was demonstrated to be above 1%. Since the maximum concentration of PHMB in cosmetic products is now reduced to 0.1%, there is a negligible risk for the consumer with respect to skin sensitization.

Dermal/percutaneous absorption

Taken together, the experimental results from the available in vitro skin penetration studies show that PHMB is a very poor skin penetrant. The physicochemical properties of the polycationic polymer PHMB are indicative of low or very low skin penetrability, i.e. it is not a pure chemical substance, but contains a range of polymeric molecules. The (mean/median) molecular weight is relatively high (>700 Da, average molecular weights are in the range between 2670 and 4216 Da) (SCCS, Ref 100, 2015), it has a high water solubility, and very low lipophilicity. In addition, the positively charged PHMB has a strong affinity to the stratum corneum by binding to the negatively charged skin surface. This view has recently been confirmed by the Danish EPA in their recent Assessment of Nano-enabled Technologies in Cosmetics, stating that *surface charge is a major contributor to avoid dermal delivery to systemic circulation due to the interaction of positively charged particles with the negatively charged skin cells*.

Results from new *in vitro* skin penetration studies with 0.1% PHMB in two representative cosmetic formulations, aqueous micellar solution and o/w emulsion, showed that the total amounts absorbed were negligible. The majority of the applied dose was detected in the “residual stratum corneum + epidermis” layer. Kinetic data on skin penetration over an extended monitoring period of 72 h demonstrate that the radiolabelled PHMB fraction present in the “residual stratum corneum + epidermis” layer does not act as a reservoir for continued percutaneous penetration and that this fraction can be reasonably excluded from the calculation

of the systemic exposure dose which is in line with the 9th revision of the SCCS Notes of Guidance.

The exclusion of the "residual stratum corneum + epidermis" fraction in the *in vitro* skin penetration study with 0.1% PHMB results in skin penetration rates of 4.09% (1.56% in dermis + 0.03% total absorbed + 2.5% *i.e.* 1 SD) in aqueous micellar solution and 1.94% (1.02% in dermis + 0.04% total absorbed + 0.88% *i.e.* 1 SD) in o/w emulsion. Since this study was fully compliant with the requested guidelines and incorporated more than one type of cosmetic formulations, only 1 SD was added as required by the SCCS Notes of Guidance.

The difference to the skin penetration rate of 7.65% from the previous submission is due to the reduced maximum use concentration of 0.1% for PHMB (*vs* 0.3%), the use of 1 SD (*vs* 2 SD), the use of 5 tape strippings (*vs* 20 tape strippings) in order to remove the stratum corneum, and the exclusion of the amounts found in the epidermis and remainders of the stratum corneum.

Repeated dose toxicity

In subacute oral studies, designed as range-finding and/or palatability studies, predominantly body weight, food and/or water consumption were affected after administration of PHMB via the drinking water for 28 days at 0, 0.1, 0.5, .10, 2.0 mg/mL in rats or 0, 0.1, 0.3, 0.6 and 1.2 mg/mL in mice. Indications of functional impairment of the liver and kidney but no serious damage of organs were noted. A NOEL was not achieved at 0.1 mg/mL (corresponding to about 16/18 mg/kg bw/day in rats, about 21 mg/kg bw/day in mice) in these studies. However, as no histopathology was performed, the relevance of the observed findings remained uncertain and therefore these were not considered as adverse effects. The reduced palatability, which could have led to secondary effects in these drinking water studies in rats and mice, indicated that this route of exposure cannot be considered as an appropriate oral route of exposure.

The subacute administration of PHMB via the diet for 6 weeks to one dog per sex at dose levels of 0, 2500, 4000 and 5500 ppm showed signs of systemic toxicity at both high dose levels. At 5500 ppm reduced food intake and weight loss in the female was observed. At 4000 and 5500 ppm there was evidence of liver toxicity characterised by reductions in plasma cholesterol, increases in the activities of specific plasma enzymes associated with liver changes and microscopic hepatocellular changes. There was also evidence for minor kidney changes at these dose levels. The NOEL for male and female dogs was 2500 ppm (corresponding to about 90 mg/kg bw/day).

The subacute dermal administration of PHMB under an occlusive dressing at dose levels of 0, 20, 60 and 200 mg/kg bw/day for 6 h/day during a 30 day period led to no sign of systemic toxicity in male and female rats. Signs of slight to moderate skin irritation occurred in a dose-dependent manner and increase with regards to severity and duration. The NOAEL for systemic toxicity was 200 mg/kg bw/day, the highest dose level investigated. The NOAEL for dermal irritation was 20 mg/kg bw/day.

The subacute inhalation of PHMB at concentrations of 0, 0.025, 0.25 or 2.5 mg/m³ for 6 hours per day, 5 days per week, over a period of 28 days led to substance-induced findings at 0.25 and 2.5 mg/m³ in the form of some transient but reversible histopathological changes in the larynx and trachea that were characteristic of exposure to a respiratory tract irritant. Body weight changes were present at these exposure concentrations. Some non-resolving histopathology changes in the lungs were limited to the highest exposure concentration of 2.5 mg/m³. The NOAEL for systemic toxicity was 0.025 mg/m³ in male and female rats under the condition of the study.

The dietary administration of PHMB to rats at dose levels of 0, 1000, 2000, 4000 and 6000 ppm over a period of up to 90 days led to a reduction in body weight at ≥2000 ppm. The kidney was identified as the target organ for animals receiving 6000 ppm. The NOAEL was 1000 ppm (corresponding to about in 84/92 mg/kg bw/day in males/females).

The dietary administration of PHMB to mice at dose levels of 0, 1000, 2000, 4000 and 6000 ppm over a period of up to 91 days caused severe toxicity at 6000 ppm. The lower dose levels of 2000 and 4000 ppm led to a clear reduction in body weight. At target organ for systemic toxicity was not identified. The NOAEL was 1000 ppm (corresponding to about in 162/224 mg/kg bw/day in males/females).

The dietary administration of PHMB to male and female Beagle dogs at dose levels of 0, 300, 1500 and 4500/3000 ppm for one year led to severe signs of toxicity at 4500/3000 ppm in the form of clinical signs of toxicity and indications of liver impairment. Treatment-related histopathological findings were present in the form of dermatitis of the scrotum, chin and limbs as well as in the liver, kidney and testis. The NOAEL for systemic toxicity was 1500 ppm (corresponding to about 54 mg/kg bw/day) in male and female Beagle dogs.

The dietary administration of PHMB at dose levels of 0, 200, 600 and 2000 ppm in a combined chronic toxicity/carcinogenicity study in male and female rats for a period of up to two years led to treatment-related findings only at the high dose of 2000 ppm in the form of slightly reduced survival in females, reduced body weight in both sexes and changes in clinical-chemistry indicative for a slight liver impairment. The NOAEL for chronic toxicity was 600 ppm (equivalent to about 36 and 45 mg/kg bw/day for males and females, respectively). The NOAEL of 36 mg/kg bw/day obtained in this two year feeding study in male animals will be used as the most representative lowest value for repeated dose toxicity for the calculation of the Margin of Safety (MoS).

Mutagenicity/Genotoxicity

PHMB was sufficiently tested for its mutagenic/genotoxic potential *in vitro* and *in vivo* covering all relevant endpoints genetic damage.

In vitro, PHMB was negative in Ames tests, did not induce gene mutations *in vitro* at the TK locus in P388 or L5178Y mouse lymphoma cells and led to no increase in chromosome aberrations in cultured human peripheral blood lymphocytes, when tested each in the absence and presence of metabolic activation up to cytotoxic concentrations.

In vivo, PHMB led to no induction of micronucleated polychromatic erythrocytes in male or female mice, when tested up to the maximum tolerated dose level and was shown to be exhibit no clastogenic potential *in vivo*.

The substance did not induce DNA-damage in the hepatocytes of the rats and was shown to be non-genotoxic in the *in vivo/ in vitro* UDS test system, when tested up to a dose level inducing overt clinical findings indicative for systemic availability and systemic toxicity.

Carcinogenicity

The carcinogenic potential of PHMB was investigated in several oral long-term studies in rats and mice as well as in a dermal skin painting study in mice.

The dietary administration of PHMB at dose levels of 0, 200, 600 and 2000 ppm in a combined chronic toxicity/carcinogenicity study in male and female rats for a period of up to two years led

to treatment-related findings only at the high dose of 2000 ppm in the form slightly reduced survival in females, reduced body weight in both sexes and changes clinical-chemistry indicative for slight liver impairment. The criteria for a maximum tolerated dose (MTD) were achieved. It can be concluded that there was no evidence of a carcinogenic effect associated with the administration of PHMB in this two year rat bioassay. The no observed effect level for chronic toxicity in the rat following dietary administration of PHMB for two years was 600 ppm (equivalent to about 36 and 45 mg/kg bw/day for males and females, respectively).

The dietary administration of PHMB in a life-time feeding study in mice at dose levels of 0, 400, 1200 and 4000 ppm led to marked toxicity at the highest dose level of 4000 ppm. This dose caused marked decreases in body weight gain and was clearly well in excess of a MTD. The mid-dose of 1200 ppm PHMB fulfilled the criteria for a maximum tolerated dose based on reduced body weight gain and non-neoplastic pathological changes at three sites (liver, recto-anal junction and gall bladder). The highest dietary concentration used, 4000 ppm, resulted in a markedly altered tumor profile. At this dose several squamous cell carcinomas of the recto-anal junction occurred in mice of both sexes and gall bladder papillomas occurred in two males. Vascular tumors, mainly haemangiosarcomas, were increased whereas lymphosarcomas were decreased in both sexes compared to the controls. Pituitary gland adenomas were also decreased in female mice. However the changes in tumor profile were considered to be of doubtful toxicological significance as the dose level exceeded by far the MTD. 1200 ppm was the maximum tolerated dose and no observed effect level for oncogenicity.

The results and assessment of the study author and study pathologist were controversial discussed and many subsequent investigations in the form of additional statistically analyses, histopathological peer reviews and comprehensive data assessment of independent experts were performed. This discussion is still ongoing and no final conclusion was drawn until now. However, the majority of the experts expressed their opinion that based on the review of all data and a weight of evidence approach, that the observed incidence of vascular tumors in the high-dose group in the mouse feeding study compared with controls is not a carcinogenic alert.

However, the European RAC did not follow this conclusion and stated that the overall evidence on carcinogenic potential of PHMB is not strong but should be classified as carcinogenic Carc 2 (CLP).

Finally, five cancer studies have been carried out with PHMB in total (each two oral studies in rats and mice, one dermal study in mice). As no statistical analysis was carried out after tumor re-assessment of tumor types in the original study reports by the PWG, statistical analysis on the PWG tumor assessment for the two key studies in rats and mice was performed.

The results from the statistical analysis on the rat study showed that no test was statistically significant at the 5% significance level (no p-value ≤ 0.05). The statistical test for a decreasing trend for haemangioma among the lymph node mesenteric neoplasms in male rats at the lowest three doses (0, 200, and 600 ppm) was statistically significant at the 6% significance level (p-value: 0.0577). This decrease in the vascular tumors observed in the mesenteric lymph nodes further reinforced the interpretation that the few non-statistically significant differences between groups occurred by chance and thus, were not related to PHMB treatment. In addition, the former rat study showed no increase in the number of vascular tumors at any site or an increase in total number of animals with vascular tumors at any dose at or below the MTD, which points to the variability between sites and studies. It has to be emphasized that interpretation of the mouse oral study should not include the top dose that excessively exceeded the MTD. Once this group was removed, no statistically significant vascular finding was observed in either the oral or dermal mouse studies, from pair wise comparisons or trend analysis.

Based upon independent expert analysis of the tumor data in the mouse and rat studies, the weight of evidence from five separate life-time cancer studies is reduced to a single dose above the MTD in the mouse oral study. However, findings at doses above the MTD are generally recognized as not relevant for the purposes of cancer classification.

Thus, based on the weight of evidence of all data, PHMB should be considered to possess no carcinogenic potential for rats or mice.

Reproduction toxicity

In a two-generation reproduction study, the dietary administration of PHMB to groups of male and female rats at dose levels of 200, 600 and 2000 ppm group led to signs of systemic toxicity in the form of decreased body weights in animals of both sexes and both generations at 2000 ppm. There was no effect on reproductive performance and fertility as well as no effect on pre- or postnatal development. The NOAEL for systemic parental toxicity was 600 ppm (corresponding to about 70 – 71mg/kg bw/day in males, 77 – 79 mg/kg bw/day in females). The NOAEL for reproductive toxicity including fertility as well as for postnatal development was 2000 ppm (corresponding to about 239 – 249 mg/kg bw/day in males, 258 – 270 mg/kg bw/day in females).

In prenatal developmental toxicity studies, no selective effects on fetal morphology were observed in rats, mice and rabbits.

The oral administration of PHMB of 0, 200, 1000 and 2000 ppm (corresponding to about 0, 13, 54, 112 mg/kg bw/day) to pregnant rats during gestational days 0 – 20 resulted in overt maternal toxicity at 1000 and 2000 ppm in the form of reduced food consumption and body weight. Prenatal developmental toxicity in terms of a slight increase in fetuses with extra ribs was observed in the high dose groups only. However, this finding coincided with significant maternal toxicity at the same dose levels and was therefore considered as secondary in nature. Thus, there was no indication of a selective effect on fetal morphology and especially no indication for teratogenicity. The NOAEL for maternal toxicity was 200 ppm (about 13 mg/kg bw/day) and for prenatal developmental toxicity was 1000 ppm (about 54 mg/kg bw).

The oral administration of PHMB at dose levels of 10, 20 and 40 mg/kg bw/day to mice from implantation to day 15 of gestation resulted in marginal signs of maternal toxicity in the form of a slightly but not statistically significant reduction in mean body weight gain at the 40 mg/kg bw/day only. There was no increase in the incidence of fetal abnormalities. However, there was a marginal retardation of ossification in single skeletal structures at 20 mg/kg bw/day and above. The NOAEL for maternal toxicity was 40 mg/kg bw and for prenatal developmental toxicity was 10 mg/kg bw/day. However, the overall validity of the study is considered to be limited due to the uncommonly very low pregnancy rate in all groups including the control.

The oral administration of PHMB at dose levels of 0, 10, 20, 40 mg/kg bw/day to pregnant New Zealand white rabbits from implantation to day 20 of gestation resulted in overt maternal toxicity at 40 mg/kg bw/day in the form of intercurrent deaths, reduced food consumption, body weight loss during treatment and one total resorption. The NOAEL for maternal toxicity was 20 mg/kg bw/day and for prenatal developmental toxicity was 40 mg/kg bw/day.

Human data

In a human insult patch test, aqueous PHMB solutions up to a concentration of 5% PHMB did not show any undesirable primary skin irritation.

PHMB is capable of causing skin sensitisation in humans after repeated occlusive exposure at induction concentrations of at least 2% PHMB. Nevertheless, in over 30 years of manufacturing PHMB at sites in the UK and the USA, no cases of dermatological problems have been reported to the occupational health unit at either site. In addition, medical surveillance information during 2004 until 2007 of manufacturing and laboratory employees examined every six months for signs of skin sensitization, showed no reported case of skin sensitization to PHMB.

Results from clinical patch tests (2.5 and 5% PHMB in water) indicated that skin sensitization from PHMB can be considered to be extremely rare.

In addition, there was no indication of photo-sensitisation in an adapted repeat insult patch test (RIPT) at a topical dose of 1% PHMB in water in male and female volunteers.

1.2 Overview of study acceptability

Study reports and relevant published data are presented in this submission according to the SCCS Notes of Guidance. The studies are considered adequate when conducted in compliance with OECD or other internationally accepted expert testing guidelines, performed according to Good Laboratory Practice (GLP) and/or the purity/composition of the test article was determined. Studies are also considered adequate when they provide additional information or relevance to the risk assessment of Polyaminopropyl biguanide (PHMB).

The following table provides an overview of the guidelines and/or GLP followed by each study reviewed in this submission. The testing guidelines cited are those in force at the time the studies were conducted. In addition, scientifically reliable studies are also listed for sake of completeness.

TEST	MATERIAL	TEST GUIDELINES	GLP	REFERENCE
ACUTE TOXICITY				
18. Oral				
19. Acute oral toxicity in rats, 2003	20. PHMB (batch: PC 100-02, solid, 96.0%)	21. OECD 425, US OPPTS 870.1100	22. Yes	23. Ref 26, 2003a
24. Acute oral toxicity in rats, 1979	25. PHMB (Vantocil P, batch: Bx791/2, ADGM 1021/79)	26. Comparable to OECD 401	27. Yes	28. Ref 63, 1979a
29. Dermal				
30. Acute dermal toxicity in rats, 2003	31. PHMB (batch: PC 100-02, solid, 96.0%)	32. OECD 402, Commission Directive 92/69/EEC, US OPPTS 870.1200	33. Yes	34. Ref 27, 2003b
35. Acute dermal toxicity in rats, 1979	36. PHMB (Vantocil P, batch: Bx791/2, ADGM 1021/79)	37. Comparable to OECD 402	38. Yes	39. Ref 63, 1979a
40. Inhalation				
41. Acute inhalation toxicity in rats, 2013	42. PHMB (batch: no data, purity: 99.6%)	43. OECD 403	44. Yes	45. Ref 2 2013
46. Acute inhalation	47. PHMB	48. OECD 403	49. Y	50. ECHA

TEST	MATERIAL	TEST GUIDELINES	GLP	REFERENCE
toxicity in rats, 2011	(batch: no data)		e s	Ref 33, 2011b
51. SKIN IRRITATION				
52. Acute dermal irritation in rabbits, 2003	53. PHMB (batch: PC 100-02, solid, 96.0%)	54. OECD 404, Commission Directive 92/69/EEC, US OPPTS 870.2500	55. Y e s	56. Ref 28, 2003c
57. Acute dermal irritation in rabbits, 1980	58. PHMB (Vantocil P, batch: Bx791/2, ADGM 1021/79)	59. Comparable to OECD 404	60. Y e s	61. Ref 65, 1980a
62. Acute dermal irritation in rabbits, 1979	63. PHMB (Vantocil P, batch: Bx791/2, ADGM 1021/79)	64. Comparable to OECD 404	65. Y e s	66. Ref 64, 1979b
67. Acute dermal irritation in rabbits, 2011	68. PHMB (batch: no data, purity: 99.6%)	69. Comparable to OECD 404	70. Y e s	71. ECHA, Ref 33 2011b
72. EYE IRRITATION				
73. Acute eye irritation in rabbits, 2003	74. PHMB (batch: PC 100-02, solid, 96.0%)	75. OECD 405, Commission Directive 92/69/EEC, US OPPTS 870.2400	76. Y e s	77. Ref 28, 2003c
78. Acute eye irritation in rabbits, 1981	79. PHMB (Vantocil IB, batch: ADGM 1021/79)	80. Comparable to OECD 405	81. Y e s	82. Ref 88, 1981
83. Acute eye irritation in rabbits, 2011	84. PHMB (batch: no data, purity: 99.6%)	85. Comparable to OECD 405	86. Y e s	87. ECHA, Ref 33 2011b
88. SKIN SENSITIZATION				
89. Guinea pig maximisation test (GPMT), 1993	90. PHMB (Vantocil P, batch: D4097)	91. OECD 406	92. Y e s	93. Ref 30, 1993
94. GPMT, 2011	95. PHMB (batch: no data)	96. OECD 406	97. Y e s	98. ECHA, Ref 33 2011b
99. GPMT, 1980	100. PHMB (Vantocil P, batch: Bx791/2, ADGM 1021/79)	101. Comparable to OECD 406	102. e s	103. Ref 66, 1980b
104. GPMT (cross-reactivity), 1983	105. PHMB (Vantocil IB, batch: ADGM 1021/79)	106. Comparable to OECD 406	107. e s	108. Ref 67, 1983b
109. Buehler test, 1980	110. PHMB (Vantocil IB , batch: Bx791/2, ADGM 1021, Vantocil IB)	111. Comparable to OECD 406	112. e s	113. Ref 66, 1980b
114. Buehler test, 1983	115. PHMB (Vantocil IB , batch:	116. Comparable to OECD 406	117. e	118. Ref 68,

TEST	MATERIAL	TEST GUIDELINES	GLP	REFERENCE
	Bx791/2, ADGM 1021, Vantocil IB)		s	1983a
119. DERMAL/PERCUTANEOUS ABSORPTION				
120. In vitro				
121. The in vitro percutaneous absorption of 0.1% radiolabelled Polyhexamethylene Biguanide (PHMB) in two representative cosmetic formulations through human skin with a monitoring time of 24 h, 2016	122. PHMB and labelled [^{14}C]-PHMB (non-labelled batch: 14GR177464; labelled batch: CFQ42591)	123. OECD 428, OECD Guidance document No. 28, SCCS Basis Criteria (SCCS/1358/10)	124. es	125. Ref 109, 2016a; Ref 77, 2016
126. The in vitro percutaneous absorption of 0.3% radiolabelled Polyhexamethylene Biguanide (PHMB) in two representative cosmetic formulations through human skin with an extended monitoring time of 72 h, 2016	127. PHMB and labelled [^{14}C]-PHMB (non-labelled batch: 14GR177464; labelled batch: CFQ42591)	128. OECD 428, OECD Guidance document No. 28, SCCS Basis Criteria (SCCS/1358/10)	129. es	130. Ref 110, 2016b; Ref 77, 2016
131. Percutaneous absorption of radiolabelled PHMB in a representative cosmetic formulation through human skin, 2014	132. PHMB and labelled [^{14}C]-PHMB (non-labelled batch: 09GR243581; labelled batch: 99BDR99)	OECD 428, OECD Guidance document No. 28, SCCS Basis Criteria (SCCS/1358/10)	133. es	134. Ref 108, 2014
135. Human epidermal penetration study, **1**996	136. PHMB and labelled [^{14}C]-PHMB (batch: no data)	137. Epidermal penetration study	138. es	139. Ref 20, 1996
140. Human epidermal penetration study at Spa temperature,	141. PHMB (batch: no data)	142. Epidermal penetration study	143. es	144. Ref 21, 1998

TEST	MATERIAL	TEST GUIDELINES	GLP	REFERENCE
1998				
145. Human epidermal and rat whole skin penetration study, 1982	146. PHMB and labelled [^{14}C]-PHMB (batch: no data)	147. Explorative epidermal and whole skin penetration study	148. o	149. Ref 31, 1982
150. Whole skin penetration study in rats, 1976	151. PHMB (batch: no data)	152. Explorative whole skin penetration study	153. o	154. Ref 69, 1976a
155. **REPEATED DOSE TOXICITY**				
156. Subacute – oral				
157. 28-day range-finding study, rats, 1992	158. PHMB (Vantocil P, batch: D4097)	159. Range-finding study	160. es	161. Ref 55, 1992a
162. 28-day range-finding study, mice, 1992	163. PHMB (Vantocil P, batch: D4097)	164. Range-finding study	165. es	166. Ref 56, 1992b
167. 6-weeks range-finding study, dogs, 1992	168. PHMB (batch: no data, 20% aqueous PHMB solution)	169. Range-finding study	170. es	171. Ref 95, 1993
172. Maximum tolerated dose study, dogs, 1992	173. PHMB (batch: no data, 20% aqueous PHMB solution)	174. Maximum tolerated dose study	175. o	176. Ref 94, 1992
7. **Subacute - dermal**				
178. 30-day dermal study, rats, 1993	179. PHMB (Vantocil P, batch: no data)	180. Comparable to OECD 410	181. es	182. Ref 76, 1993
183. 21-day dermal study, rats, 1972	184. PHMB (Vantocil IB, batch: no data)	185. Screening study	186. o	7. Ref 36, 1972
188. **Subacute - inhalation**				
189. 5-day range-finding inhalation study, rats, 2006	190. PHMB (Vantocil IB, batch: Bx6142)	191. Range-finding study	192. es	193. Ref 89, 2006a
194. 5-day inhalation study with 13-week recovery, rats, 2006	195. PHMB (Vantocil IB, batch: Bx6142)	196. Range-finding study	197. es	198. Ref 90, 2006b
199. 28-day inhalation study with 13-week recovery in rats, 2006	200. PHMB (Vantocil P, batch: no data)	201. OECD 412, Council Directive 67/548/EEC, OPPTS 870.3465	202. es	203. Ref 91, 2006c
204. 21-day inhalation study	205. PHMB (Vantocil IB, batch: no	206. Explorative inhalation	207. o	208. Ref 16.,

TEST	MATERIAL	TEST GUIDELINES	GLP	REFERENCE
in, rats, 1976	data)	study		1976
209. **Subchronic - oral**				
210. 90-day oral (diet) range-finding study in rats, 1993	211. PHMB (Vantocil P, batch: D4097)	212. Range-finding study with an extent comparable to OECD 408	213. es	214. Ref 58, 1993a
215. 13-week oral (drinking water) palatability study in mice, 1993	216. PHMB (Vantocil P, batch: D4097)	217. Palatability study	218. es	219. Ref 57, 1992c
220. 91-day dietary sighting study in mice, 1993	221. PHMB (Vantocil P, batch: D4097)	222. Range-finding study with an extent comparable to OECD 408	223. es	224. Ref 59, 1993c
225. 90-day dietary range-finding study in dogs, 1966	226. PHMB (batch: WEM/G/680)	227. Range-finding study	228. o	229. Ref 48, 1966b
0. **Chronic – oral**				
231. Combined chronic toxicity/carcinogenicity study in rats, 1996	232. PHMB (Vantocil P, batch: D4097)	233. Comparable to OECD 453	234. es	235. Ref 61, 1996, Ref 84, 1993, Ref 13, 1996, Ref 11, 2002a
236. 1-year oral (diet) study in dogs, 1995	237. PHMB (Vantocil P, batch: D4097)	238. Comparable to OECD 452	239. es	240. Ref 60, 1995; Ref 84, 1993
241. MUTAGENICITY/GENOTOXICITY: *IN VITRO*				
242. Bacterial reverse mutation test (Ames), 1989	243. PHMB (Vantocil IB, batch: Bx791/2, ADGM 1021)	244. OECD 471	245. es	6. Ref 14, 1989
247. Bacterial reverse mutation test (Ames), 1979	248. PHMB (Vantocil IB, batch: AGDM 2253/77)	249. Comparable to OECD 471	250. o	1. Ref 51, 1979
252. Bacterial reverse mutation test (Ames), 1979	253. PHMB (Vantocil IB, batch: AGDM 2253/77)	254. Comparable to OECD 471	255. o	6. Ref 111, 1980
7. Mammalian cell gene mutation test, 2002	258. PHMB (P20D, batch: 1202)	259. OECD 476	260. es	1. Ref 50, 2002

TEST	MATERIAL	TEST GUIDELINES	GLP	REFERENCE
2. Mammalian cell gene mutation test, 1981	263. PHMB (Vantocil P, batch ADGM/1021/79)	264. OECD 476	265. es	6. Ref 1 1981
267. Mammalian cell chromosomal aberration test, 1989	268. PHMB (Vantocil IB, batch: Bx2125)	269. OECD 473	270. es	1. Ref 62, 1989
272. Mammalian cell chromosomal aberration test, 1989	273. PHMB (Vantocil P, batch: ADGM/1021/79)	274. OECD 473	275. es	6. Ref 98, 1981
7. **MUTAGENICITY/GENOTOXICITY: *IN VIVO***				
278. Mammalian micronucleus test, mice, 1989	9. PHMB (Vantocil IB, batch: Bx2125)	280. OECD 474	281. es	2. Ref 97, 1989
283. UDS assay, rats, 1989	4. PHMB (Vantocil IB, batch: Bx2125)	285. Comparable to OECD 486	286. es	7. Ref 112, 1989
8. CARCINOGENICITY				
9. Oral studies				
290. Combined chronic toxicity/carcinogenicity study in rats, 1996	291. PHMB (Vantocil P, batch: D4097)	292. Comparable to OECD 453	293. es	294. Ref 61, 1996, Ref 84, 1993, Ref 13, 1996, Ref 11, 2002a
295. Combined chronic toxicity/carcinogenicity study in rats, 1977	296. PHMB (Baquacil SB, batches: SDC/596 and ADGM 5911)	297. Comparable to OECD 453	298. o	299. Ref 4, 1977
300. Carcinogenicity study in mice, 1996	301. PHMB (Vantocil P, batch: D4097)	302. US EPA Guideline 83-2, Comparable to OECD 451	303. es	4. Ref 87, 1996 Ref 84, 1993, Ref 12, 2002b
305. Carcinogenicity study in mice, 1977	306. PHMB (Baquacil SB, batches: SDC/596, ADGM 5911)	307. Comparable to OECD 451	308. o	9. Ref 19, 1977b
0. Demal studies				
311. 80-week skin painting study in mice, 1977	312. PHMB (Baquacil SB, batches: SDC/596, ADGM 5911)	313. Skin painting study prior to establishment of specific testing guidelines	314. o	5. Ref 18, 1977a
6. REPRODUCTION TOXICITY				
7. Fertility and reproduction				

TEST	MATERIAL	TEST GUIDELINES	GLP	REFERENCE
318. Two-generation reproduction toxicity study in rats, 1995	319. PHMB (Vantocil P, batch: D4097)	320. Comparable to OECD 416	321. es	2. Ref 86, 1995, Ref 84, 1993
323. Three-generation reproduction toxicity study in rats, 1995	324. PHMB (batch: no data)	325. Explorative three-generation reproduction toxicity study	326. es	7. Ref 113, 1990
3. Prenatal developmental toxicity				
329. Prenatal developmental toxicity study in rats, 1976	0. PHMB (Baquacil SB, batch: no data)	331. Comparable to OECD 414	332. o	3. Ref 54, 1976
334. Prenatal developmental toxicity study in mice, 1977	5. PHMB (Baquacil SB, batch: no data)	336. Comparable to OECD 414	337. o	3. Ref 53, 1977, Ref 39, 40, 1981a, b
339. Prenatal developmental toxicity study in rabbits, 1993	340. PHMB (Vantocil P, batch: D4097)	341. Comparable to OECD 414	342. es	3. Ref 9, 1993b
344. Prenatal developmental toxicity study in rabbits, 1976	345. PHMB (batch: no data)	346. Explorative developmental toxicity study	347. o	3. Ref 3, 1976
9. TOXICOKINETICS AND METABOLISM				
350. Absorption, distribution, metabolism and excretion screening study in rats, 1995	351. PHMB and labelled [^{14}C]-PHMB (batch: no data)	352. Explorative absorption, distribution, metabolism and excretion screening	353. es	4. Ref 80, 1995a
355. Absorption screening study in mice, 1995	356. PHMB and labelled [^{14}C]-PHMB (batch: no data)	357. Explorative absorption, screening	358. es	9. Ref 70, 1976b
360. Bioavailability screening in rats, 1995	361. PHMB and labelled [^{14}C]-PHMB (batch: no data)	362. Explorative bioavailability screening	363. es	4. Ref 79, 1995b
365. Gastro-intestinal absorption screening in rats, 1975	366. PHMB and labelled [^{14}C]-PHMB (batch: no data)	367. Explorative gastro-intestinal absorption screening in rats,	368. o	9. Ref 10, 1975
370. HUMAN DATA				
371. Irritation				
372. Human insult patch test	373. PHMB (Cosmocil CQ, batch: No.	374. Insult patch test	375. e	376. Ref 107,

TEST	MATERIAL	TEST GUIDELINES	GLP	REFERENCE
(HIPT), 2001	0237)	according to standards set by Japanese patch test study group	s	2001
377. Sensitization				
378. Human repeated insult patch test (HRIPT), 1981	379. PHMB (Vantocil IB, batch: no data)	380. HRIPT according to the method of Shelanski, 1951, Shelanski and Shelanski, 1953 and Stotts, 1980	381. e s	382. R ef 104, 1981
383. Photo-sensitization				
384. Human modified repeated insult patch test (HRIPT) with direct rays of mid-day sun, 1976	385. PHMB (Baquacil SB, batch: ADGM 3429)	386. Explorativ e photo-sensitization screening test	387. e s	388. R ef 52, 1976

2. Introduction

After comprehensive review of all available data on Polyaminopropyl Biguanide (PHMB) the ECHA Committee for Risk Assessment (RAC) came to the conclusion that there is no strong evidence for a carcinogenic potential. The RAC emphasised that Category 2 is recommended, if there is limited evidence of carcinogenicity.

As a consequence, the RAC concluded that "*Data suggest a carcinogenic effect but are limited for making a definitive evaluation because e.g. a) the evidence of carcinogenicity is restricted to a single experiment b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential or d) the evidence is restricted to studies that demonstrates only promoting activity in narrow range of tissues or organs.*

With respect to PHMB, the evidence of carcinogenicity (systemic and local) is mainly from a single experiment (mouse oral carcinogenicity study), but there is supporting evidence from other studies in mice (criteria (a) is valid). There are remaining uncertainties about interpretation with respect to the MTD (criteria (b) is valid). PHMB is not genotoxic in vitro and in vivo, but taking into account that the overall evidence on carcinogenicity is mainly on the evidence from one study in one species and no mode of action has been identified, a classification as carcinogenic Carc 2 – H351 (CLP) and category 3; R40 (DSD) is warranted" (references: ECHA, Ref 32, 33, 2011a, b).

Article 15 of the Regulation (EC) No. 1223/2009 (recast of the so-called Cosmetics Directive) stated that "*The use in cosmetic products of substances classified as CMR substances, of category 2, under Part 3 of Annex VI to Regulation (EC) No 1272/2008 shall be prohibited. However, a substance classified in category 2 may be used in cosmetic products where the substance has been evaluated by the SCCS and found safe for use in cosmetic products. To these*

ends the Commission shall adopt the necessary measures in accordance with the regulatory procedure with scrutiny referred to in Article 32(3) of this Regulation."

This updated dossier on PHMB

- represents an evaluation of the toxicological profile in accordance with the recent SCCS opinion (SCCS, Ref 100, 2015)
- demonstrates that the risk for human health with respect to carcinogenic, mutagenic or reprotoxic effects (CMR) of PHMB is negligible from the use of cosmetic products
- supports its safe use as preservative in cosmetic products at a maximum use concentration of 0.1%.

3. Abbreviations

2-AA:	2-aminoanthracene
4-NPD:	4-nitro-O-phenylenediamine
6-BT:	6-p-dimethylaminophenyylazobenzthiazole
ALP:	Alkaline phosphatase
ALT:	Alanine transaminase
ANOVA:	Analysis of variance
AST:	Aspartate transaminase
BHK21:	Baby hamster kidney fibroblasts
Bq:	Becquerel (units of radioactivity)
BrdU:	5-bromodeoxyuridine
bw:	Body weight
CAS:	Chemical Abstracts Service
CLP:	(EU Regulation on) Classification, Labelling and Packaging
cm:	centimeter
cm^2:	square centimeter
CPA:	Cyclophosphamide
DKG:	German Contact Dermatitis Research Group
DMEM:	Dulbecco's Modified Eagle Medium
DMSO:	Dimethylsulphoxide
DNA:	Deoxyribonucleic acid
dpm:	Disintegrations per minute
DR:	Daunorubicin
DSD:	Dangerous Substances Directive
EC:	European Commission
ECHA:	European Chemicals Agency
ELISA:	Enzyme Linked Immunosorbent Assay
EPA:	Environmental Protection Agency
EU:	European Union
f:	female
FCA:	Freund's complete adjuvant
FDA:	Food and Drug Administration (USA)
g:	gram
GBq:	Giga Bequerel
GD:	Gestational day
GHS:	Globally Harmonized System (of Classification and Labeling of Chemicals)

GI:	Gastrointestinal (tract)
GLP:	Good Laboratory Practice
GSD:	Geometric standard deviation
H&E:	Hematoxylin & eosin (staining)
h:	hour
HCD:	Historical control data
HIPT:	Human insult patch test
HMBDA:	Hexamethylenebisdicyandiamide
HMD:	Hexamethylenediamine
HPLC:	High performance liquid chromatography
HRIPT:	Human repeat insult patch test
i.e.:	id est (Latin) = that is
i.p.:	intraperitoneal
ICDRG:	International Contact Dermatitis Research Group
IL-6:	Interleukin-6
INCI:	International Nomenclature of Cosmetic Ingredients
KC1:	Potassium chloride
kg:	kilogram
Kp:	Permeability coefficient
L:	Liter
LC_{50}:	Lethal concentration for 50% of the population
LD_{50}:	Lethal dose for 50% of the population
LOAEL:	Lowest observed adverse effect level
LOQ:	Limit of quantification
LPS:	Lipopolysaccharide
LSC:	Liquid scintillation counting
M or m:	male or molar, molarity
m^3:	cubic meter
mCi:	millicuries
mg:	milligram, 10^{-3} gram
mL:	milliliter
mm:	millimeter
mM:	millimolar
MMAD:	Mass medium aerodynamic diameters
MMC:	Mitomycin C
MMS:	Methyl methanesulfonate
MNNG:	N-methyl-N-nitro-N-nitrosoguanidine
MoS:	Margin of Safety
MPE:	Micronucleated polychromatic erythrocytes
MTD:	Maximum tolerated dose
MTT:	Methylthiazole tetrazolium
MTT:	3-(4,5-dimethylthiazol-2-lyl)-2,5diphenyl tetrazolium
N or n:	Number (sample size)
ng:	nanogram
nM:	nanomol
NOAEC:	No observed adverse effect concentration
NOAEL:	No observable adverse effect level
NOAEL:	No observed adverse effect level
NOEC:	No observed effect concentration

NOEL:	No observed effect level
NOG:	Notes of guidance
ns:	not significant
NTP:	National Toxicology Program
NZW:	New Zealand White (rabbit)
OECD:	Organization for Economic Co-operation and Development
OPPTS:	Office of Prevention, Pesticides and Toxic Substances (US Environmental Protection Agency)
p.c.:	post coitum
PCE:	Polychromatic erythrocytes
PHMB:	Poly(hexamethylene) biguanide hydrochloride or Polyaminopropyl Biguanide
PII:	Primary irritation index
ppm:	Parts per million
PWG:	Pathology Working Group
QUA:	Quality Assurance Unit
RAC:	Committee for Risk Assessment
ROS:	Reactive oxygen species
RSG:	Relative suspension growth
RTG:	Relative total growth
SAP:	Scientific advisory panel
SC:	*Stratum corneum*
SCCS:	Scientific Committee on Consumer Safety
SD:	Standard deviation
SED:	Systemic exposure dose
TFT:	Trifluorothymidine
TK:	Thymidine kinase
TNF:	Tumor necrosis factor
UDS:	Unscheduled DNA synthesis
y:	year
µCi:	micro Curie
µg:	microgram; 10^{-6} gram
µM:	micromole

4. Chemical and physical specification

4.1 Chemical identity

4.1.1 Primary chemical name/INCI name

Chemical name: Poly(hexamethylene) biguanide hydrochloride

INCI name: Polyaminopropyl Biguanide

(SCCS, Ref 100, 2015)

4.1.2 Chemical names

IUPAC Name: Homopolymer of N-(3-Aminopropyl)-Imidodicarbonimidic Diamide

Other chemical names:
Poly(hexamethylenebiguanide hydrochloride)
Poly(iminocarbonimidoyliminocarbonimidoylimino-1,6-hexanediyl), hydrochloride
Poly(iminoimidocarbonyl-iminoimidocarbonyl-iminohexamethylene), hydrochloride
Poly(iminoimidocarbonyliminoimidocarbonyliminohexamethylene) hydrochloride

(SCCS, Ref 100, 2015)

4.1.3 Trade names and abbreviations

Baquacil
Cosmocil CQ
PHMB
Polihexanide
Polyhexanide
Vantocil IB
Vantocil TG

(SCCS, Ref 100, 2015)

4.1.4 CAS/EINECS Numbers

CAS numbers: 32289-58-0 / 27083-27-8 / 28757-47-3 / 133029-32-0

Two equivalent CAS number can be allocated depending on how the polymer is described.

CAS-No 27083-27-8 expresses the PHMB in terms of its starting monomers (N,N’’’-1,6-hexanediylbis(N’-cyanoguanidine) and 1,6-hexanediamine).

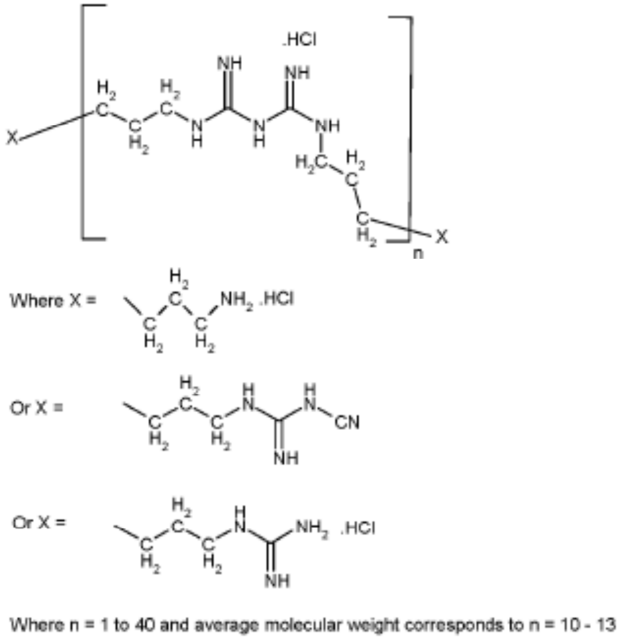
CAS-No 32289-58-0 expresses the PHMB as the resultant polymer.

EC: Not allocated as the substance is a polymer (the substance was not notified under Directive 92/32/EEC)

(SCCS, Ref 100, 2015)

4.1.5 Structural formula

Structural formula:



(SCCS, Ref 100, 2015)

4.1.6 Empirical formula

$(C_8H_{17}N_5)_n.nHCl$, n=1 -40

(SCCS, Ref 100, 2015)

4.2 Physical form

Off-white to pale yellow powder with strong ammonia smell at 20° C and 101.3 kPa
Very pale yellow to pale yellow, lumpy solid; no obvious odor.
Pale-yellow glass-like solid (technical grade PHMB).

(SCCS, Ref 100, 2015)

4.3 Molecular weight

Molecular weight: > 700 g/mol
2670 – 2960 (weight average molecular weight) Da (Ref 5, 2003)
3686 – 4216 (weight average molecular weight) Da (Ref 24, 2008)

For Vantocil P it is described that on average (5 samples tested) 5.3 % is present as monomers, i.e. as constituent with a molecular weight below 500 Da (Ref 41, 1991)

Radiolabelled PHMB: 7% molecular weight fraction < 500 Da, 10% molecular weight fraction 500 to 1000 Da, 83% molecular weight fraction > 1000 Da (Ref 77, 2016)

4.4 Purity, composition and substance codes

Degree of purity: >94.2% (w/w in dry weight)

(ECHA, Ref 33, 2011b; Ref 41, 1991, 1994; Ref 5, 2003; Ref 24, 2008; SCCS, Ref 100, 2015)

4.5 Impurities / accompanying contaminants

Impurities:

Redacted as CBI

4.6 Solubility

Water solubility: 41 ± 1 % [w/w] at 25° C (Ref 2, 2013)
39.0 – 43.4 % at 23.4° C (Ref 5, 2003)
426 g/l (Ref 108, 2014)

Solubility in organic solvents: Methanol: 41 ± 1 % w/w at 25 ± 1°C
Ethanol: 0.5 ± 0.08 % w/w at 25 ± 1°C
Acetone: 2.7 ppm at 22°C
Dichloromethane: 0.2 ppm at 22°C
Ethyl Acetate: 0.1 ppm at 22°C
Toluene: 0.2 ppm at 22°C
n-hexane: 0.1 ppm at 22°C
Acetonitrile: 0.8 ppm at 22°C

(ECHA, Ref 33, 2011b)

4.7 Partition coefficient (Log Pow)

Log P_{ow}: -2.3 at 25 ± 1° C; pH: 7.4 (determined as approx. 20 % aqueous solution according to OECD TG 107 (shake-flask method))

(Ref 6, 2007)

4.8 Additional physical and chemical specifications

Melting point: a) 78.9- 136.3° C
b) decomposes without melting at 205 – 210 °C

Boiling point: decomposes at 205-210°C before boiling

Vapour pressure: a) 1.32 x 10-7 Pa (20°C) and 4.11 x 10-7 Pa (25°C)
(estimated according to OECD TG 104)
b) 6.0 x 10-8 Pa (20°C) and 2.0 x 10-7 Pa (25°C)
(estimated according to OECD TG 104)

Relative Density: 1.20 ± 0.0025 (at 20 ± 0.5°C)

pKa: 4.19 at 25°C

pH: 4.36 at 21.7° C

UV_Vis spectrum: wavelength maximum at 236 nm

Surface tension: 68.5±0.6 mN/m temperature: 25±0.5° C

Hydrolysis: hydrolytically stable as only <10% hydrolysis after 5 days for pH 4, 7 and 9 at 50°C (OECD guideline 111)

Photolysis: PHMB is considered as non photodegradable as significant light absorption above the 290 nm cut-off of solar irradiation was not found in visible wavelength (OECD guideline 316 criterion)

(ECHA, Ref 33, 2011b; Ref 17, 2008; McNab, 2002; Ref 5, 2003; SCCS, Ref 100, 2015)

4.9 Stability

Shelf life: at least 2 years

Accelerated storage stability: stable over a period of 14 days at 54±2 °C (Ref 5, 2003)

The stability of PHMB in deionised water has been established for at least 6 weeks (Ref 84, 1993)

Nominal concentrations (expressed as Vantocil, i.e. 20 % PHMB) of 0.1, 35 and 80 mg/ml were reported to be stable for the duration of the test (Ref 30, 1993)

Stability analysis revealed that PHMB over a concentration range of 0.02 to 7.0 mg/l was stable in drinking water for a period of 7 days (Ref 55, 56, 57, 1992)

5. Function and uses

5.1 Cosmetic product preservation

Cosmetic products on the market have to be safe for their intended uses. This includes the effective preservation of cosmetic products which is a requirement under the EU Cosmetics Regulation (Regulation (EC) No 1223/2009).

Cosmetics do not need to be sterile. However, they must not be contaminated with pathogenic microorganisms, and the density of non-pathogenic microorganisms should remain low. Preservatives are incorporated into cosmetic products to prevent the growth of microorganisms that could adversely affect the health of the consumers (Ref 45, 2006). Without preservatives, bacteria, yeast, mould and other organisms could develop, leading to product deterioration, spoilage and potential health and safety issues.

The Cosmetics Regulation in Europe has a limited list of permitted preservatives. This regulation requires i) that cosmetic products are protected from microbial contamination to avoid consumer health issues, and ii) that preservatives used in cosmetics are safe by undergoing a safety evaluation by the SCCS (Scientific Committee for Consumer Safety) and regulatory provisions. SCCS i) evaluates the safety profile of preservatives used in cosmetics and ii) gives some guidance on microorganism limits and efficient preservation testing (SCCS, Ref 101, 2016).

An increasing number of cosmetic products are recalled each year, the majority being contaminated with potential pathogenic micro-organisms. *Pseudomonas aeruginosa* is the most frequently identified microorganism in recalled cosmetic products (Ref 78, 2008). This issue clearly demonstrates the need for adequately preserved cosmetics.

In particular, water-based personal care products provide conditions for growth of a wide range of microorganisms that may be introduced during use by the consumer, in particular when products are packaged in multiple use containers. Multiple use containers are favored by consumers and they have a lower cost compared to single use cosmetic products.

5.2 Importance of keeping a wide palette of efficient and safe preservatives

Many preservatives are under scrutiny from either a regulatory, safety or public perception standpoint. Over the past years, a continuous trend was observed to reduce the number of preservatives allowed for use in cosmetic products while new preservatives are hardly developed, mainly due to the ban of animal testing.

An adequate preservation of products would be impossible without a sufficiently wide palette of authorized preservatives, especially in case of water-based products which are most susceptible to microorganism growth. Individual cosmetic formulations and products need individual preservation. Without efficient, safe and cost effective preservation options, microbial contamination of those products may result in an increase in health issues.

A wide variety of preservatives is important for the safety of the consumer in order to avoid increased exposure to only a few of them thereby increasing the risk of safety issues such as sensitisation.

To summarize, it is important to maintain a broad range of preservatives:

i) to adequately protect products from microorganism contamination and ensure the microbial safety of cosmetics to consumers,

ii) to continue to provide products which are safe and meet the needs and expectations of consumers, keeping in mind that multiple use containers are favored by a number of consumers and that single use containers are increasing the cost of cosmetic products,

iii) to avoid the over-exposure of consumers to one preservative which may increase the risk of safety issues.

5.3 Properties and uses of preservatives

Substances allowed for preservative use in cosmetic products are presented in a positive list in the EU Cosmetics Regulation EC N°1223/2009 (Annex V). Formulators of cosmetic products have thus to select among authorized substances when developing preservation systems for their products.

However, the choice of preservatives that may be used in a specific cosmetic product is driven by a number of criteria. These criteria include:

1. Efficacy: - from product manufacture to reasonably foreseeable consumer use conditions - preferably against a broad spectrum of microorganisms
2. Compatibility: - with all other ingredients that are present in the product and packaging -with the galenics of the formulation -with physical properties, color, odor… of the product
3. Stability: - over ranges of temperature, pH etc. covering product use
4. Safety: - preservative(s) in the product should remain below levels evaluated as safe
5. Others: - analytical determination of preservative level in the product should be possible for quality and in-market control
   - cost should be taken into account

Among substances listed in Annex V, only those fulfilling the above-mentioned criteria may be adequate candidates for the preservation of cosmetic products. This shows that only a limited number of preservatives can in practice be used to protect cosmetic products and consumers.

5.4 Properties and uses of PHMB

Polymers based on biguanides (polybiguanides) such as PHMB are polycationic amine derivatives composed of cationic biguanide repeat units separated by aliphatic chains. These polycationic polymers are highly substantive and bind by electrostatic interactions to surfaces with negatively charged groups such as fabrics (carboxylic groups in cellulose fibres) (Ref 114, 2011), bacterial membranes, and the skin surface. The net charge of human skin is negative and thus, the positively charged PHMB will bind strongly to the negatively charged skin surface. As PHMB is polycationic the binding with a surface is at multiple sites per molecule. In order for the PHMB to be desorbed it is required that the binding at all sites is released simultaneously, kinetically this is unlikely and this "polychelating effect" is demonstrated by the high substantivity of PHMB. Likewise, the bactericidal mode of action of PHMB particularly includes attachment by electrostatic attraction of the positively charged biguanide groups to the universally negatively charged outer surface of bacteria (Ref 72, 2010; Ref 85, 1999; Ref 96, 2016).

PHMB is used as a preservative in cosmetic formulations and found on the market at a maximum use concentration of 0.1%.

At the concentrations used in **cosmetic formulations**, PHMB is a gentle, non-irritating and non-sensitizing fast acting, broad spectrum preservative. It has an excellent activity against a wide range of Gram positive and Gram negative bacteria, fungi and yeasts and is particularly effective against difficult to control microorganisms such as *Pseudomonas species*. It is notably effective amongst the following bacteria of concerns: *Pseudomonas aeruginosa*, *Staphylococcus aureus.*

Table 5.4.1: Antimicrobial spectrum (Ref 71, 1984)

Test organisms ($\approx 10^6$ CFU/mL)	Minimal inhibitory concentration (µg/mL) (serial dilution test ; incubation times of 24 and 72 h)
Staphylococcus aureus	389. 20
Streptococcus faecalis	5
Escherichia coli	20
Pseudomonas aeruginosa	100
Aspergillus niger	375

PHMB is one of the few remaining preservatives which are globally accepted in cosmetic formulations. It is freely water-soluble and therefore widely used in water-based products where amphiphilic preservatives are not compatible. It is stable over a wide range of pH and temperatures as well as UV stable. PHMB is one of the few preservatives effective at neutral pH, unlike other water-soluble preservatives (i.e. organic acids). Thus, PHMB is an effective preservative used notably for eye and face make-up removers, moisturizing toners, facial cleanser, wipes, skin care products, hair dyes and hair care products, since it has been shown to be not irritating to the eyes and better tolerated than other preservatives.

The use of PHMB in non-propellant driven spray products (pump sprays) is included in the scope of the applicants portfolio. As the safety of the final cosmetic product belongs to the responsibility of the manufacturer, marketer or the responsible person, the applicants will take care that in formed aerosols from PHMB containing cosmetic non-propellant driven spray products, the droplets to which consumers may be exposed will have sizes of above 10 µm. It is generally accepted that droplets of >10 µm are trapped, impacted and/or filtered in the nose, mouth, throat or tracheobronchial area and will not reach the deeper parts of the lungs.

Content redacted as EU specific

6. Toxicological evaluation

6.1 Acute toxicity

6.1.1 Acute oral toxicity

Study Design:

Reference:	Ref 26, 2003a
Date of report:	2003
Guideline/method:	OECD 425, US OPPTS 870.1100
Species/strain:	Rat/Sprague-Dawley
Group size:	6 female rats
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	PC 100-02 (solid, 96.0%)
Dose levels:	550, 2000 mg/kg bw
Vehicle:	Distilled water
Application volume:	20 mL/kg bw
Route:	Oral (gavage)
Exposure:	Single application
Observation period:	14 days
GLP:	Yes
Published:	No

Material and methods:

PHMB was administered by a single oral administration to a total of six fastened female Sprague-Dawley rats in sequence with at least 48 hours between the animals at dose levels of 2000 mg/kg bw or 550 mg/kg bw. PHMB was solved in distilled water and applied at an application volume of 20 mL/kg bw. The animals were observed for treatment-related effects for a 14-day observation period. The body weight was monitored. At termination, the animals were sacrificed and a gross pathological examination was conducted.

Results:

At 2000 mg/kg bw all three animals were found dead during the day of dosing or one day thereafter. No deaths occurred at 550 mg/kg bw. Unspecific clinical signs were recorded only at 2000 mg/kg bw, which consisted of hunched posture, piloerection, lethargy, ataxia, decreased respiration rate, laboured respiration, ptosis and tiptoe gait. The surviving animals gained body weight. Abnormalities noted at necropsy of animals that died during the study were haemorrhagic or abnormally red lung, dark liver, dark kidneys, haemorrhage or sloughing of the gastric mucosa, sloughing of the non-glandular epithelium of the stomach and haemorrhage of the small intestine. No abnormalities were noted at necropsy in animals that survived through the 14-day observation period.

Conclusion:

The oral LD_{50} of PHMB was 1049 g/kg bw in female Sprague-Dawley rats under the conditions of the study. In an earlier study, PHMB as 20% aqueous solution (Vantocil P, batch: Bx791/2, ADGM 1021/79) was administered by oral administration to each of 5 fastened Alderley Park, SPF-derived albino rats per sex at dose levels of 700, 1000, 1500, 2000, 2500, 3000, 3500, 5000 mg/kg bw. PHMB was diluted in deionized water and applied at an application volume of 10

mL/kg bw. The animals were observed for treatment-related effects for a 14-day observation period. There occurred dose-dependently increased mortalities at ≥2000 mg/kg bw, in general within the first 6 hours after application. Only unspecific clinical signs were recorded, which consisted of salivation, lacrimation, piloerection and in isolated cases, a subdued appearance. These signs did not persist beyond day 7 or 8 of the study. The oral LD_{50} of the 20% aqueous PHMB solution was 2747 mg/kg bw in males and 2504 mg/kg bw in females (equivalent to 549 mg PHMB/kg in males or 501 mg PHMB/kg in females; Ref 63, 1979a).

6.1.2 Acute dermal toxicity

Study Design:

Reference:	Ref 27, 2003b
Date of report:	2003
Guideline/method:	OECD 402, Commission Directive 92/69/EEC, US OPPTS 870.1200
Species/strain:	Rat/Sprague-Dawley
Group size:	5 male and 5 female rats
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	PC 100-02 (solid, 96.0%)
Dose levels:	5000 mg/kg bw
Vehicle:	moistened with distilled water
Application area:	≥10% of total body surface
Route:	Dermal (semi-occlusive)
Exposure:	Single application
Observation period:	14 days
GLP:	Yes
Published:	No

Material and methods:

PHMB was tested for its acute dermal toxicity in a limit test in 5 male and 5 female Sprague-Dawley rats. The animals were dermally exposed to a single dose of 5000 mg/kg bw of the test substance moistened with distilled water to the clipped skin (dorsal and dorso-lateral parts of the trunk) and covered by semi-occlusive dressing for 24 hours. The application area comprised at least 10% of the total body surface area. Body weights were determined shortly before administration (day 0) and on days 7 and 14. Clinical signs were recorded several times on the day of administration and at least once daily thereafter. The skin was examined and the findings were scored according to Draize (1977) 0.5, 1, 2 and 4 hours after removal of the semi-occlusive dressing (day 1) and subsequently once per week for 14 days. After the observation period had been completed, animals were sacrificed and gross pathological examination was conducted.

Results:

No mortality occurred and no signs of systemic toxicity were observed. The dermal application led to signs of dermal irritation in the form of very slight to well defined Erythema, hemorrhage of dermal capillaries and small superficial scattered scabs. The body weight gain was not affected. No abnormalities were noted at necropsy.

Conclusion:

The acute dermal toxicity (LD_{50}) was >5000 mg/kg bw for male and female Sprague-Dawley rats. In an earlier study performed according to the method of Draize (1959), PHMB as 20% aqueous solution (Vantocil P, batch: BX 791/2, ADGM 1021/79) was occlusively applied to the

skin of male and female New Zealand white rabbits for 24 hours. All animals were shaved prior to application and in each 2 males and 2 females the skin was abraded using a scalpel blade. PHMB was applied as received at a standard volume of 2 mL to the backs of each rabbit. At the end of the 24 hour period the dressings were removed, the skin cleansed with water and the animals were observed for 14 days. No deaths occurred in male and female rabbits and thus, the dermal LD_{50} of the 20% aqueous PHMB solution was >2 mL/kg bw (about >400 mg PHMB/kg bw) under the conditions of the study (Ref 63, 1979a). The same dermal LD_{50} value of >400 mg/kg bw for rats was reported in the ECHA background document after single dermal application of a 20% aqueous PHMB solution (ECHA, Ref 33, 2011b).

6.1.3 Acute inhalation toxicity

Information on the acute inhalation toxicity of PHMB is available in the CHL proposal by the French authority. PHMB (purity 99.6%) as aqueous solution was studied in a GLP, OECD 403-compliant acute inhalation toxicity study in male and female Wistar rats. The animals were nose-only exposed to an aerosol of PHMB. Mass medium aerodynamic diameters were in the range of 1.49-2.20 µm with geometric standard deviation (GSD) in the range of 1.84-2.29 µm. In a preliminary study, both animals exposed at 1.0 mg/L died after 1 and 2 hours of exposure respectively. Severely laboured respiration was observed and dark red, diffuse discoloration of enlarged or non collapsed lungs with foamy white content in trachea was observed at necropsy. At 0.1 mg/L, no lethality occurred, slight to moderate clinical signs were observed (laboured respiration, rhonchus, partial ptosis, decreased activity, increased respiratory rate, sneezing). Transient body weight decrease was observed but no test-item related macroscopic findings. Based on these results, concentrations in the main study were 0.1, 0.3 and 0.5 mg/L PHMB for 4 hours for each five male and five female rats. No animal died at 0.1 mg/L, 3/5 males died at 0.3 mg/L and all male animals and 3/5 females died at 0.5 mg/L. Clinical signs in the form of laboured respiration, rhonchus, decreased activity, hunched back and increased respiration rate occurred at a concentration-dependent increased severity at all concentration but improved during the post observation period. Initially, animals lost body weight but the body weight gain improved during the cause of the exposure free period. Necropsy revealed an enlargement of dark/red discoloured lungs and/or dark/red discoloration of the fur at the perinasal and/or white foamy material in the trachea were seen in all animals found dead at 0.3 and 0.5 mg/L. No PHMB exposure related macroscopic observations were noted in animals exposed to concentrations up to 0.5 mg/L at terminal sacrifice. The LC_{50} was 0.29 mg/L/4 h for males, 0.48 mg/L/4 h for females and 0.37 mg/L/4 h for males and females combined (1.85 mg/L for 20%-PHMB solution; Ref 2, 2013).

This is in line with another reference on the acute inhalation toxicity of a formulation containing 20.6 (% w/w) PHMB. Alpk:APfSD (Wistar derived) rats (five/sex) were exposed by nose-only for 4 hours to a single dose of 1.76 mg/L of the formulation corresponding 0.36 mg/L of PHMB (mass medium aerodynamic diameters (MMAD) were 1.8 - 2.0 µm with a GSD of 2 µm). Three hours after the exposure one male out of ten died. All females and most males revealed respiratory impairments including breathing irregularities and abnormal respiratory noise. Red mottled lungs were found in the dead male, as well as two other males on day 15. The LC_{50} was >0.36 mg/L/4 h for PHMB (ECHA, Ref 33, 2011b)

6.1.4 Overall conclusion on acute toxicity

The acute toxicity of PHMB can be considered as moderate to low. The acute oral LD_{50} of PHMB determined in a Guideline conform study under GLP conditions was 1049 mg/kg bw in female Sprague-Dawley rats. In an older study with lower reliability the oral LD_{50} of the 20% aqueous PHMB solution was 2747 mg/kg bw in male and 2504 mg/kg bw in female (equivalent

to 549 mg PHMB/kg bw in males or 501 mg PHMB/kg bw in females) Alderley Park, SPF-derived albino rats. The acute dermal LD_{50} of PHMB determined in a Guideline conform study under GLP conditions was >5000 mg/kg bw for male and female Sprague-Dawley rats. In an older study with lower reliability the dermal LD_{50} of the 20% aqueous PHMB solution was >2 mL/kg bw in rabbits (about >400 mg PHMB/kg), which is the same value reported in the ECHA background document with another source. There is information on the acute inhalation toxicity of PHMB in the CHL proposal by the French authority. According to this reference, the LC_{50} was 0.29 mg/L for males, 0.48 mg/L for females and 0.37 mg/L for males and females combined (1.85 mg/L for 20%-PHMB solution). This is in line with another reference in the RAC document, which cited that the LC_{50} was >0.36 mg/l for PHMB in male and female Alpk:APfSD (Wistar derived) rats. From the clinical signs observed the acute inhalation toxicity of PHMB is considered to be caused more by local than by systemic effects.

As supported by market data from the Mintel database, exposure of consumers from inhalation is insignificant. Based on current and anticipated use conditions as preservative in cosmetic preparations up to a maximum concentration of 0.1%, PHMB can be considered as safe for users of cosmetic products.

6.2 Irritation and corrosivity

6.2.1 Skin irritation

Study Design:

Reference:	Ref 28, 2003c
Date of report:	2003
Guideline/method:	OECD 404, Commission Directive 92/69/EEC, US OPPTS 870.2500
Species/strain:	Rabbit/New Zealand White
Group size:	3 male animals
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	PC 100-02 (solid, 96.0%)
Dose level:	0.5 g neat substance moistened with 0.5 ml distilled water
Route:	intact shorn skin (about 6.25 cm²), semi-occlusive dressing
Duration:	4-hour
Skin readings:	1, 24, 48, 72 hours, day 7
Observation period:	7 days
GLP:	Yes
Published:	No

Material and methods:

The skin irritation potential of PHMB was investigated in rabbits. An amount of 0.5 g of neat PHMB moistened with 0.5 mL distilled water was applied to the shorn skin of 3 rabbits at an area of 6.25 cm² and an adjacent area of untreated skin served as control. Each skin site was covered with a semi-occlusive dressing. One animal was initially treated with a patch on three sites on the back. One of these patches was removed after 3 minutes, 1 and 4 hours after application. After consideration of the skin reactions of the first animal, two other animals were treated with each one patch under semi-occlusive conditions for 4 hours. All animals were observed for sign of skin irritation (erythema, edema) at 1, 24, 48 and 72 h after patch removal and thereafter once daily up to day 7. Skin reactions were scored according to the OECD guideline method.

Results:

Following 3 minutes and one hour of exposure, no evidence of skin irritation was noted. After 4 hours of exposure, well-defined erythema at one treated skin site with very slight erythema at two treated skin sites one and 24 after patch removal were noted. Very slight erythema was noted at all treated skin sites at 48-hour observation and persisted at one site until the 72 hour observation. Slight edema was noted at one treated site one hour after patch removal with very slight edema at 24- and 48-hour observation. Tow treated skin sites appeared normal at the 72-hour observation and one treated skin site appeared normal at the 7-day observation. No corrosive effect was noted. The primary irritation index was 1.0

Conclusion:

Under the conditions of the study, neat PHMB was mildly irritating to the skin of rabbits.

In a previous skin irritation study with lower reliability, a 20% aqueous PHMB solution (Vantocil P, batch: BX 791/2,(ADGM 1021/79) was tested for its skin corrosivity potential in male New Zealand White rabbits. The application to the material as supplied to the intact and abraded skin of six animals led to superficial scabbing and erythema around the abrasions but there were no signs of necrosis to the intact skin of any of the animals. Thus, it was considered as non-corrosive to the rabbit skin (Ref 64, 1979b).

In another previous study, the primary skin irritation potential of a 20% aqueous PHMB solution (Vantocil P, batch: BX 791/2, ADGM 1021/79) was examined in a group of six female New Zealand white rabbits. The animals received the material as supplied on the intact and abraded skin of the flanks at an area of about 6.25 cm² for 24 hour under an occlusive dressing. After patch removal the skin was examined at 24 and 72 hours for erythema and edema and the skin irritation was assessed using the Draize method. In a separate section of the study, three male animals were treated with PHMB according to the same scheme but one animal was killed at 48 hours and skin samples were taken for histopathological examination. The remaining two animals were killed at 72 hours and skin samples were taken for histopathological examination. Signs of marked to severe skin irritation were noted clinically and the primary irritation index (PII) was 2.6. The skin irritation was confirmed by histopathology showing moderate to marked acute skin inflammation. Based on the results of this study, the authors concluded that PHMB was a moderate irritant to the intact skin but severe irritation was caused when applied to the abraded skin of male and female rabbits (Ref 65, 1980a).

In more recent studies, the application of solid PHMB (96%) to the skin of rabbits according to OECD 404 induced no signs of skin irritation but after dermal application of a 20% aqueous PHMB solution moderate erythema was recorded 24 hours after application on the treated area of all three animals. The reaction was completely reversible between days 6 and 8. No oedema was observed with the 20% aqueous solution (ECHA, Ref 33, 2011b).

6.2.2 Mucous membrane irritation

Study Design:

Reference:	Ref 28, 2003c
Date of report:	2003
Guideline/method:	OECD 405, Commission Directive 92/69/EEC, US OPPTS 870.2400
Species/strain:	Rabbit/New Zealand White
Group size:	1 male animal in contrast to initially planned 3 male animals due to severity of observed effects
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	PC 100-02 (solid, 96.0%)
Dose level:	0.1 mL neat substance

Route:	instillation in the conjunctival sac of the right eye
Readings:	1, 24, 48, 72 hours, days 7, 14, 21 after instillation
Observation period:	up to 21 days
GLP:	Yes
Published:	No

Material and methods:

The potential irritant effect of PHMB was investigated by instillation of 0.1 mL of the neat test substance into the conjunctival sac of one eye of the animal, the untreated eye served as control. Initially, one rabbit was used. The eyes were not rinsed. Both eyes were examined at 1, 24, 48 and 72 h as well as on days 7, 14 and 21 after instillation. The effects on the cornea, iris and conjunctivae were scored according to the OECD guideline method. Due to the severity of the ocular response, no additional animal was treated for animal welfare reasons.

Results:

The single application to the non-rinsed eye of the animal led to opalescent corneal opacity, iridial inflammation and severe conjunctival irritation. Other ocular effects were noted in the form of dulling of the normal corneal lustre, vascularisation and pale appearance of the nictitating membrane. Translucent corneal opacity, minimal conjunctival irritation and vascularisation were noted at the 21-day observation and were considered as irreversible.

Conclusion:

Under the conditions of the study, neat PHMB induced irreversible ocular damage and was considered as corrosive to the rabbit eye.

In a previous eye irritation study with lower reliability, a 20% aqueous PHMB solution (Vantocil IB, batch: ADGM 1021/79) was examined in nine female New Zealand White rabbits. About 0.1 mL of the test substance as supplied was instilled in the conjunctival sac of one eye, the other eye remained untreated and served as control. The eyes of six animals were not irrigated after instillation. The eyes of the other three animals were irrigated for one minute with lukewarm water about 20 – 30 seconds after the instillation. The animals were examined and the grade of ocular reaction was recorded at 1 – 2 h and 1, 2, 3, 4, 7, 8, 15, 25, 26, 28 and 35 days after instillation according to the method of Draize (1959). Most of the animals showed signs of slight to moderate initial pain following instillation. The animals where the eyes were not rinsed showed iritis and conjunctivitis and 4/6 animals showed corneal opacity, which recovered by day 25. The animals with the rinsed eyes showed conjunctivitis and one had slight iritis, but none of them showed any corneal reaction (Ref 88, 1981).

In a more recent study, PHMB supplied as powder (purity 99.6%) was instilled into the eye of one New-Zealand rabbit at the dose of 0.1 g. At the conjunctival level, a moderate redness was noted 1 hour after instillation and still noted at the end of the observation at day 7. It was associated with chemosis noted 24 hours after instillation and lasting until the end of the observation. At the corneal level, a moderate opacity was registered 1 hour after instillation, which persisted until the end of the observation period. Iris congestion was registered from the 2nd day and persisted until the end of the observation. An ulceration of the nictating membrane and the cornea was noted from the 1st day and this lesion persisted for 72 hours (ECHA, Ref 33, 2011b).

6.2.3 Overall conclusion irritation

PHMB tested as neat compound in a Guideline conform study under GLP conditions was mildly irritating to the skin of rabbits. In more recent studies, the application of solid PHMB to the skin

of rabbits induced no signs of skin irritation but dermal application of a 20% aqueous PHMB solution caused moderate but completely reversible erythema.

When neat PHMB was examined for its eye irritation potential in a Guideline conform study under GLP conditions, the compound caused irreversible ocular damage and was considered as corrosive to the rabbit eye. In a more recent study, PHMB supplied as powder induced chemosis and a persistent moderate opacity was noted for the cornea. Persistent iris congestion was recorded and an ulceration of the nictating membrane and the cornea was noted persisting for 72 hours.

Finally, considering that PHMB is used as preservative in cosmetic products on the market only up to 0.1%, it can be concluded that there is no risk of skin or eye irritation for the consumer from these exposures.

6.3 Skin sensitization

6.3.1 Guinea pig maximization tests

Study Design:

Reference:	Ref 30, 1993	
Date of report:	1993	
Guideline/method:	OECD 406 (Magnusson and Kligman, 1970)	
Species/strain:	Guinea pig/Dunkin Hartley	
Group size:	20 females in the test group, 10 control group females	
Test substance:	Polyaminopropyl biguanide (PHMB)	
Batch:	D4097 (Vantocil P, 20.2% aqueous PHMB solution)	
Vehicle:	Deionised water	
Positive control:	2-mercaptobenzothiazole (3% preparation in corn oil for intradermal induction and topical challenge, 75% preparation in corn oil for topical induction)	
Concentrations:	Intradermal induction:	3% solution in deionized water, with/without Freund's complete adjuvant (FCA), controls only vehicle with/without FCA
	Dermal induction:	1 week after intradermal induction supplied material (100%) fixed by occlusive dressing for 48 h
	Challenge:	2 weeks following dermal induction Supplied material (100%) and 30% solution in deionized water fixed by an occlusive dressing for 24 h
Readings:	24, 48 h after patch removal according to a four point scale	
GLP:	Yes	
Published:	No	

Material and methods:

The sensitizing potential of PHMB (Vantocil P, batch: D4097, 20.2% aqueous PHMB solution) was tested in 30 female Dunkin Hartley guinea pigs in the maximization test following the published method of Magnusson and Kligman (1970). Prior to the main study, range finding studies with intradermal injection and topical application (induction and challenge) were performed using 2 – 4 animals to determine tolerable and non-irritant concentrations for the main study. Based on the results of the range finding studies the animals were intradermally injected

with 0.1 ml of a 3% aqueous solution with or without Freund's complete adjuvant into the shaven shoulder region of each 30 animals. One week after the induction injection, a topical booster patch with the undiluted material was occlusively applied on the induction site for 48 hours. The challenge was performed by occlusive epicutaneous application of the undiluted material and a 30% solution in deionized water to a previously untreated site for 24 hours. The application sites were scored 24 and 48 hours after removal of the patch according to the scheme of Draize. The sensitivity of the Guinea pigs was investigated with 2-mercaptobenzothiazole as positive control.

Results:

Following challenge with the undiluted material, scattered mild redness or moderate diffuse redness was observed in the 18/20 of test animals at 24 h and 16/20 at 48 h (average scores of 1.4 at 24 h and 1.2 at 48 h). Scattered mild redness was observed in 4/10 of the control animals at 24h and 2/10 at 48h. The net frequency of response at 24h was 50%.

Following challenge with the 30% solution, scattered mild redness or moderate diffuse redness was observed in 5/20 test animals at 24 h and 2/20 at 48 h (average scores of 0.3 at 24 h and 0.1 at 48 h) and scattered mild redness was observed only in 1 of the ten control animals at 24 h.

The sensitivity of the test animals was confirmed as the challenge of previously induced Guinea pigs with a 3% w/v preparation of 2-mercaptobenzothiazole elicited a strong skin sensitisation response.

Conclusion:

It was demonstrated that PHMB exhibited a skin sensitizing potential in Guinea pigs in the Maximization test according to Magnusson and Kligman test conditions.

Another Guinea Pig Maximisation Test was performed according to OECD 406 and GLP on 20% aqueous PHMB diluted in physiological saline. Intradermal induction was performed with 0.15% PHMB and topical induction with 20% PHMB. Challenge was performed with 20% or 10% PHMB. 24 hours after challenge moderate erythema was observed in one animal out of 10 at the 20% challenge treatment and for one animal out of 10 at the 10% concentration site in the test group. No reactions were evident in the control group. 48 hours after challenge moderate erythema was observed in one animal at the 10% treatment site in the test group and no reactions were evident in the control group. Under the conditions of this study, PHMB was not considered as a dermal sensitizer according to classification criteria (ECHA, Ref 33, 2011b).

A previous study according to Magnusson and Kligman was performed with PHMB (Vantocil IB, batch: Bx791/2, ADGM 1021, 20% aqueous PHMB solution) according to OECD 406 with the exception that no SLS was applied during induction although no signs of irritation during induction were reported. Intradermal induction was performed with 0.2% PHMB in deionised water and Freund's complete adjuvant (FCA) and topical induction with a neat preparation of the test article (20.2% PHMB). Challenge was performed with the neat test sample (20.2% PHMB). Challenge of test and control guinea pigs resulted in signs of mild to moderate erythema in 14 out of 20 test animals and mild erythema in 1 out of 8 controls at 24 hours (net frequency of response of 57.5%). At 48 hours mild to moderate erythema was present in 15 out of 20 test animals and mild erythema was still present in 1 control animal (net frequency of response of 62.5%). Although 1 control showed signs of skin irritation, the test material should be considered as having caused moderate to strong skin sensitisation under the conditions of this study (Ref 66, 1980b).

The possible cross-reactivity of PHMB (Vantocil IB, batch: ADGM 1021, 20% aqueous PHMB solution) with chlorohexidine gluconate was also examined in a study according to the

Magnusson and Kligman method. The design was comparable to OECD 406 with the exception that no SLS was applied during induction period. Intradermal induction was performed with 0.25% PHMB in water and topical induction with 20% PHMB. Challenge was performed with 20% PHMB or 0.5%, 0.5% and 4% of chlorohexidine gluconate. The challenge of test and control guinea pigs with 20% PHMB resulted in skin findings in 8 out of 20 test animals and in 3 out of 8 control animals. No cross reactivity with chlorohexidine gluconate was observed. Re-challenge with 20% PHMB resulted in positive skin reactions in 3 out of 20 test animals. Thus, it was concluded that PHMB led to mild signs of skin sensitization in the Guinea pigs under the conditions of this study (Ref 67, 1983b).

6.3.2 Buehler test

Study Design:

Reference:	Ref 66, 1980b	
Date of report:	1980	
Guideline/method:	Comparable to OECD 406	
Species/strain:	Guinea pig/Alderly Park, SPF-derived albino	
Group size:	Test group: 10 females Control group: 10 females	
Test substance:	Polyaminopropyl biguanide (PHMB)	
Batch:	Bx791/2 (ADGM 1021, Vantocil IB, 20% aqueous PHMB solution)	
Vehicle:	Distilled water	
Concentrations:	Induction:	topical application of 0.4 mL freshly prepared 10% aqueous solution (test group) or water (control group) for 6 h on 400 mm² clipped skin under an occlusive dressing, 3times/week for 3 weeks (10 applications in total)
	Rest period:	2 weeks
	Challenge:	14 days following last induction, challenge with 10% aqueous PHMB solution (0.4 mL, 400 mm²) for 6 h under occlusion on a previously unexposed site
	Re-challenge:	1, 10, 20% aqueous solution (0.4 mL, 400 mm²) for 6 h under occlusion on a previously unexposed site
Readings:	Induction: after removal of dressing and prior to application Challenge: 24, 48 h after challenge treatment Re-challenge: 24, 48, 96 h after last challenge	
GLP:	Yes	
Published:	No	

Material and methods:

The sensitizing potential of PHMB (Vantocil IB, batch: Bx791/2, ADGM 1021, 20% aqueous PHMB solution) was tested in Alderly Park SPF-derived albino Guinea pigs in the Buehler test comparable to OECD guideline 406 under GLP condition. In total 20 female animals were examined, 10 test group animals and 10 control group animals. Prior to the first application, the

scapular region was clipped free of hair. This area was treated with a topical application of 0.4 mL freshly prepared 10% aqueous solution (test group) or water (control group) for 6 h on 400 mm² skin under an occlusive dressing, 3times/week for 3 weeks (10 applications in total). The skin was examined for signs of irritation after removal of each patch and before application of the next patch. The animals were then left untreated for two weeks after the last induction. Thereafter, both flanks of each animal were clipped free of hair and the animals were challenged by topical application 10% aqueous PHMB solution (0.4 mL, 400 mm²) for 6 h under occlusion on a previously unexposed site. The skin reaction was read using a four-point scale 24 and 48 hours after the removal of the dressing. A re-challenge was performed 7 days later with a 1, 10 or 20% aqueous solution under comparable conditions and assessed at 24, 48 and 96 hour after removal of the patch.

Results:

Challenge resulted in signs of faint erythema in 6 out of 10 test animals at 48 hours but there were no signs of erythema in any of the control animals. Rechallenge with the 20% solution resulted in faint to moderate erythema in 8 out of 9 test animals and 3 out of 10 controls. Rechallenge with a 10% solution resulted in faint erythema in 3 out of 10 test animals, but not in controls. Rechallenge with a 2% solution did not cause an erythematous response in either test or control animals.

In conclusion, a 2% solution of PHMB is a moderate to strong sensitizer to guinea pig skin under the conditions of the study. New control animals were used for comparison with the re-challenged animals.

Conclusion:

It was demonstrated that PHMB tested as 10% aqueous solution was a moderate skin sensitizer in female Guinea pigs under the conditions of this study. The lack of a response with the 1% solution at re-challenge was considered as suggestive of a challenge dose-response relationship.

Thereafter, the effect of variation in induction and challenge concentrations of PHMB (Vantocil IB, batch: ADGM 1021, 20% aqueous PHMB solution) on skin sensitization was tested in groups of 10 males and/or 10 female Alderly Park SPF-derived albino Guinea pigs under comparable conditions as described above. The exception was that various concentrations for the induction and challenge periods were examined as follows:

Study 1	**Study 2**	**Study 3**	**Study 4**	**Study 5**	**Study 6**	**Study 7**
Induction: 0.3%	390. ndu ctio n: 0.8%	391. ndu ctio n: 1.3%	392. ndu ctio n: 1.8%	393. I nducti on: 2.0%	394. I nducti on: 1.2%	395. I nducti on: 5.0%
396. Cha enge: 0.03% 0.075% 0.15% 0.3%	397. hall eng e: 0.08% 0.2% 0.4% 0.8%	398. hall eng e: 0.13% 0.325% 0.65% 1.3%	399. hall eng e: 0.18% 0.45% 0.9% 1.8%	400. C hallen ge: 2.0%	401. C hallen ge: 1.2%	402. C hallen ge: 15.0%
				403. R e-challe nge:	404. R e-challe nge:	405. R e-challe nge:

				2.0% 15.0%	1.2% 15.0%	2.0% 1.2%

Results:

Studies 1 - 4:

Apart from slight yellow stains at some skin sites in individual animals, no abnormalities and no signs of erythema were observed at any skin site throughout the studies 1 - 4y. Thus, the induction concentrations of 0.3% - 1.8% PHMB did not led to skin sensitization in the animals.

Study 5:

During the induction phase, four animals had small scabs which appeared after the fourth application of the test material. Small scabs were also observed in four other animals by the end of the induction period. Challenge was carried out at two sites, one with lint and rubber, the other with lint and Blenderm®. No signs of erythema were observed except in one animal which had faint erythema on the lint and Blenderm® application site. Rechallenge was carried out with only lint and rubber patches and no response was observed to the concentration of 2.0% PHMB.

However, six animals responded to challenge with 15.0% PHMB. Thus, about 60% of the examined animals were induced by the induction concentration of 2.0% PHMB but a positive skin reaction was only provoked by the highest challenge concentration of 15.0% PHMB, while no skin reaction was noted using the challenge concentration of 2.0% PHMB.

Study 6:

After induction with 1.2% PHMB, challenge with 1.2% PHMB was carried out at two sites, one with lint and rubber, the other with lint and Blenderm®. No signs of erythema were observed at any application site. Rechallenge was carried out with only lint and rubber patches and no response was observed to 1.2% PHMB. Two test animals responded to rechallenge with 15% PHMB but the skin reaction was only visible three days following the challenge application. Thus, the induction concentration of 1.2% PHMB elicited a weak sensitisation reaction in the animals.

Study 7:

Moderate scabbing with some desquamation and skin thickening was observed by the end of the induction phase using 5% PHMB. For unknown reasons, two animals were found dead following the first challenge. Challenge with 15.0% PHMB was carried out at two sites, one with lint and rubber, the other with lint and Blenderm®. All of the eight surviving animals showed signs of erythema when challenged under rubber and six when challenged under Blenderm®. Rechallenge was carried out with only lint and rubber and six out of eight responded to 2% PHMB and four to 1.2% PHMB. Thus, the induction concentration of 5.0% PHMB led to strong skin reactions indicative for sensitization in the animals.

Conclusion:

In the initial series of experiments using lint and rubber dressings to determine the maximum non-sensitising concentration, results indicated no evidence of sensitisation in guinea pigs induced with 0.3, 0.8, 1.3 and 1.8% PHMB.

An induction concentration of 5% PHMB clearly sensitised guinea pigs. When an inducing concentration of 2% PHMB was re-tested, a significant response incidence was obtained when animals were challenged with 15% PHMB. Induction with.1.2% PHMB resulted in a weak response to challenge with 15% PHMB. Therefore, in the following study, animals were sensitised with 1.2%, 2% and 5% PHMB. However, the latter two studies results indicated a

differential in the concentration of PHMB that may sensitise with repeated exposure and the concentration that may elicit a response at a single challenge exposure.

Finally, it can be concluded that that the threshold concentration of PHMB, which led to sensitization in Guinea pigs is approximately 1% under the conditions of these studies. PHMB was shown to elicit clear sensitising skin reactions above an induction concentration of 1.2% (Ref 68, 1983a).

6.3.3 Overall conclusion on skin sensitization

PHMB was sufficiently investigated for its sensitizing potential in reliable adjuvant and non-adjuvant tests in experimental animals, predominantly according or comparable to guideline procedures and under GLP conditions. Several studies investigated the skin sensitising potential of PHMB in guinea pigs. In some maximisation tests a mild response was reported consistent with an absence of classification for skin sensitisation, whereas PHMB induced moderate to strong responses in other maximisation studies. The discrepancy in the results can not entirely be explained by the level of exposure to PHMB. Sensitisation was also observed in the Buehler test with repeated inductions. Investigation of dose response showed that responses of moderate to strong potency were induced from an induction concentration of 1.2% PHMB in these studies.

Overall, the positive responses observed in several studies indicate that PHMB is a skin sensitizer in animals. The threshold concentration of PHMB, which led to sensitization in Guinea pigs is approximately 1%.

Finally, considering that PHMB in cosmetic products is used as preservative up to 0.1% only, it can be assumed from the animal data that there is only a very low risk of skin sensitization, if any, for the consumer.

Table 6.3.3.1 Overview on sensitization studies with PHMB in animals

Species	**Method**	**Induction concentration**	**Challenge concentration and net frequency of response**	**Result**	**Reference**
Guinea pig	406. axi mi-sati on	407. ID: 0.06%	408. Topical: 20% or 6% PHMB: 15% 20% PHMB: 50%	409. 6% PHMB: not sensitising 20% PHMB: sensitising; strong potency	410. R ef 30, 1993
411. G uinea pig	412. axi mi-	413. ID: 0.2% Topical:	414. 20% PHMB: 62.5%	415. Sensitising; strong potency	416. R ef 66, 1980b
417. G uinea pig	418. ueh ler test	419. To pical: 2%	420. 2% PHMB: 60% Rechallenge 4%: 59% Rechallenge 2%: 30% Rechallenge 0.2%: 0%	421. Sensitising: strong potency Sensitising: strong potency Sensitising: moderate potency Not sensitising	422. R ef 66, 1980b

Species	Method	Induction concentration	Challenge concentration and net frequency of response	Result	Reference
423. Guinea pig	424. Buehler test	425. Topical: 0.3- 5%	426. 0.03-15% PHMB	427. Sensitising. Moderate to strong reaction were observed at concentration of induction ≥1.2% and challenge ≥15% or at concentration of induction ≥5% and	428. Ref 68, 1983a
429. Guinea pig	430. axi mi-	431. ID: 0.25% Topical:	432. 20% PHMB: 2.5% Rechallenge 20%: 15%	433. Not sensitising.	434. Ref 67, 1983b
435. Guinea pig	436. axi mi-sati on	437. ID: 0.15% Topical: 20%	438. 20% PHMB: 10% 10% PHMB: 10%	439. Not sensitising.	440. ECHA, Ref 33, 2011b
441. ID: intradermal					

6.4 Dermal/percutaneous absorption

6.4.1 Dermal/percutaneous absorption *in vitro*

Percutaneous absorption study through human skin *in vitro*: 0.1% PHMB in two representative cosmetic formulations with an exposure time of 24 h

Study Design:

Reference:	Ref 109, 2016; Ref 77, 2016
Study period:	2015/2016
Date of report:	2016
Guideline/method:	OECD 428, OECD Guidance document No. 28, SCCS Basis Criteria (SCCS/1358/10)
Test system:	Human skin
Total number of donors:	4 (25-50 years old)
Replicates:	Test Preparation 1: 12 (total dosed and used for calculation) Test Preparation 2: 12 (total dosed), 11 (total used for calculation)
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	a) non-radiolabelled: 14GR177464, purity: 20% b) radiolabelled: CFQ42591, [14C]-PHMB, specific activity: 51.5 mCi/g
Purity (radiolabelled):	19.06 % radiochemical purity (HPLC)
Concentration:	Test Preparation 1: 0.1% (target, 0.11% actual) Test Preparation 2: 0.1% (target, 0.10% actual)
Formulations:	Test Preparation 1: Aqueous micellar solution (composition provided in the report) Test Preparation 2: Oil in water emulsion (composition provided in the report)
Dose level:	1.97 mg/cm² (for both preparations)
Exposed area:	3.14 cm²
Skin samples:	Human split skin samples (200 – 400 µm, stored in a freezer at – 20 °C, thawed prior to use) obtained from full-thickness human abdominal skin
Skin temperature:	The skin surface temperature was maintained at 32±1 °C.
Skin integrity:	Tested by electrical resistance barrier integrity test
Test chamber:	Static diffusion cell system
Receptor fluid:	Phosphate buffered saline (PBS), containing sodium azide (0.01%, w/v).
Route:	topical application
Exposure time:	24 h
Sampling:	pre-dose, 1, 2, 4, 6, 8, 12, 24 h
Tape strips:	*Stratum corneum* was removed with 5 successive tape strips
GLP:	Yes
Published:	No

Material and methods:

The percutaneous absorption of radiolabelled [^{14}C]-PHMB (batch: CFQ42591, 51.5 mCi/g) in two representative cosmetic formulations was tested *in vitro* using human split skin samples. Split-thickness skin samples (200 – 400 µm) from 4 donors prepared from frozen, full thickness skin samples were used for the study. Split-thickness skin samples were stored at -20°C in a freezer before use. Samples were mounted on to a static diffusion cell and – after exclusion of skin samples not fulfilling integrity requirements - dosed with [14C]-PHMB at 0.1 % [w/w] in two representative cosmetic formulations, i.e. an aqueous micellar solution (Test Preparation 1)

and an oil in water emulsion (Test Preparation 2). The tested cosmetic formulations were selected to exemplify a broad range of cosmetic products containing PHMB. A total of 12 samples were assessed for each formulation. A temperature of 32 ± 1°C was maintained in the system, the application rate was 1.97 mg/cm2 for each formulation (target: 2.0 mg/cm2). Receptor fluid was collected prior to dosing and 1, 2, 4, 6, 8, 12 and 24 h after dosing. At the last sampling point, skin samples were washed with an aqueous solution of polysorbate 20 (2%, w/v) and water, dried and removed from the apparatus. The outer layers of the stratum corneum were removed by 5 tape strippings. Epidermis and dermis were then heat separated and all skin samples were analysed by liquid scintillation counting. The limit of reliable measurement was 30 disintegrations per minute (dpm).

Results:

A total of 12 samples of human split skin obtained from 4 different donors were dosed topically with [14C] PHMB in the two cosmetic formulations at a concentration of 0.1% (w/w). For the final determination of the dermal absorption, all results from the 12 samples obtained from 4 different donors were considered for Test Preparation 1 (aqueous micellar solution). For Test Preparation 2 (oil in water emulsion), only the results from 11 samples obtained from 4 different donors were considered due to low mass balance for one sample.

In Test Preparation 1 (aqueous micellar solution) and Test Preparation 2 (oil in water emulsion), 48.43% and 52.36%, respectively, was removed during the washing procedure (total dislodgeable dose). At 24 h post dose, the absorbed dose was 0.03% (0.58 ng equiv/cm2) and 0.04% (0.72 ng equiv/cm2) of the applied dose, respectively. The “epidermis + lower layers of stratum corneum” contained 11.47% (238 ng equiv/cm2) and 14.20% (291 ng equiv/cm2) of the applied dose, respectively. The dermis contained 1.56% (32.3 ng equiv/cm2) and 1.02% (20.9 ng equiv/cm2) of the applied dose, respectively. The mass balance was complete (90.93% and 98.96% of the applied dose, respectively).

Table 6.4.1.1 Skin penetration results for 0.1% [14C]-PHMB in two representative cosmetic formulations with an exposure time of 24 h (expressed as % of applied dose)

Test Preparation	Test Preparation 1 Aq micellar soln	Test Preparation 2 O/W emulsion
Target [^{14}C]-PHMB Concentration in Test Preparation (%, w/w)	442. 0.10	443. 0.10
444. Actual [^{14}C]-PHMB Concentration in Test Preparation (%, w/w)	445. 0.11	446. 0.10
447. Target Application Rate of Test Preparation (mg/cm^2)	448. 2	449. 2
450. Actual Application Rate of Test Preparation (mg/cm^2)	451. 1.97	452. 1.97
Total Number of Donors	4	4
Total Number of Replicates Dosed	12	12
Total Number of Replicates Contributing to Mean ±	12	11

SD Data		
453. (% Applied Dose)	454. (Mean ± SD)	455. (Mean ± SD)
456. Total Dislodgeable Dose*	457. 48.43 ± 8.32	458. 52.36 ± 24.18
459. Upper Stratum Corneum (Tapes 1-5)	460. 29.33 ± 5.46	461. 31.27 ± 16.19
462. Unabsorbed Dose **	463. 77.88 ± 6.58	464. 83.70 ± 11.12
465. Epidermis + Lower Layers of Stratum Corneum	466. 11.47 ± 5.69	467. 14.20 ± 8.07
468. Dermis	469. 1.56 ± 2.49	470. 1.02 ± 0.84
471. Receptor Fluid	472. 0.01 ± 0.01	473. 0.02 ± 0.03
474. Receptor Chamber Wash	475. 0.02 ± 0.01	476. 0.02 ± 0.01
477. Absorbed Dose ***	478. 0.03 ± 0.01	479. 0.04 ± 0.04
480. Mass Balance	481. 90.93 ± 2.11	482. 98.96 ± 5.44

* Total dislodegeable dose = skin wash + tissue swab + pipette tip + donor chamber wash

** Unabsorbed dose = total dislodgeable dose + upper stratum corneum (Tapes 1-5) + unexposed skin

*** Absorbed dose = receptor fluid + receptor wash

Notes

Receptor Chamber Wash = all data was below the Limit of Reliable Measurement (LoRM).

Test Preparation 1 = one out of twelve samples of 24 h Receptor Fluid was above LoRM. All other samples were below LoRM. Mean and SD were calculated from all samples (n=12) for each time point.

Test Preparation 2 = four out of twelve samples of 24 h Receptor Fluid were above LoRM. All other samples were below LoRM. Mean and SD were calculated from 11 of 12 samples for each time point.

Conclusion:

[14C] PHMB in the two test preparations was topically applied to human skin in vitro at a concentration of 0.1% (w/w). Under the present experimental conditions for [14C] PHMB in Test Preparation 1 (aqueous micellar solution) and Test Preparation 2 (oil in water emulsion), 48.43% and 52.36%, respectively, were removed during the washing procedure (total dislodgeable dose). At 24 h post dose, the absorbed dose was 0.03% (0.58 ng equiv/cm^2) and 0.04% (0.72 ng equiv/cm^2) of the applied dose, respectively. The “epidermis + lower layers of

stratum corneum" contained 11.47% (238 ng equiv/cm^2) and 14.20% (291 ng equiv/cm^2) of the applied dose, respectively. The dermis contained 1.56% (32.3 ng equiv/cm^2) and 1.02% (20.9 ng equiv/cm^2) of the applied dose, respectively. The mass balance was complete (90.93% and 98.96% of the applied dose, respectively).

Percutaneous absorption study through human skin *in vitro*: 0.3% PHMB in two representative cosmetic formulations with an exposure time of 24h and a monitoring period of 72 h

Study Design:

Reference:	Ref 110, 2016; Ref 77, 2016
Study period:	2015/2016
Date of report:	2016
Guideline/method:	OECD 428, OECD Guidance document No. 28, SCCS Basis Criteria (SCCS/1358/10), with extended monitoring period of 72 h
Test system:	Human skin
Total number of donors:	4 (25-50 years old)
Replicates:	Test Preparation 1 and 2: 12 (total dosed and used for calculation)
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	a) non-radiolabelled: 14GR177464, purity: 20% b) radiolabelled: CFQ42591, [14C]-PHMB, specific activity: 51.5 mCi/g
Purity (radiolabelled):	19.06 % radiochemical purity (HPLC)
Concentration:	Test Preparation 1: 0.3% (target, 0.34% actual) Test Preparation 2: 0.3% (target, 0.32% actual)
Formulations:	Test Preparation 1: Aqueous micellar solution (composition provided in the report) Test Preparation 2: Oil in water emulsion (composition provided in the report)
Dose level:	Test Preparation 1: 1.96 mg/cm² Test Preparation 2: 2.00 mg/cm²
Exposed area:	3.14 cm²
Skin samples:	Human split skin samples (200 – 400 µm, stored in a freezer at – 20 °C, thawed prior to use) obtained from full-thickness human abdominal skin
Skin temperature:	The skin surface temperature was maintained at 32±1 °C.
Skin integrity:	Tested by electrical resistance barrier integrity test
Test chamber:	Static diffusion cell system
Receptor fluid:	Phosphate buffered saline (PBS), containing sodium azide (0.01%, w/v).
Route:	topical application
Exposure time:	24 h
Monitoring time:	72 h
Sampling:	pre-dose, 1, 2, 4, 6, 8, 12, 24, 48, 72 h
Tape strips:	*Stratum corneum* was removed with 5 successive tape strips
GLP:	Yes
Published:	No

Material and methods:

The study incorporated a standard 24 h dosing period followed by an extended monitoring period of 48 hrs post-dosing, giving a total monitoring period of 72 h. The aim being to follow the penetration behaviour of PHMB through the dermis and epidermis over an extended time period

to demonstrate no longer term migration to the dermis or receptor fluid, ie no reservoir effect was present.

The percutaneous absorption of radiolabelled [^{14}C]-PHMB (batch: CFQ42591, 51.5 mCi/g) in two representative cosmetic formulations was tested *in vitro* using human split skin samples. Split-thickness skin samples (200 – 400 µm) from 4 donors prepared from frozen, full thickness skin samples were used for the study. Split-thickness skin samples were stored at -20°C in a freezer before use. Samples were mounted on to a static diffusion cell and – after exclusion of skin samples not fulfilling integrity requirements - dosed with [14C]-PHMB at 0.3 % [w/w] in two representative cosmetic formulations, i.e. an aqueous micellar solution (Test Preparation 1) and an oil in water emulsion (Test Preparation 2). The tested cosmetic formulations were selected to exemplify a broad range of cosmetic products containing PHMB. A total of 12 samples were assessed for each formulation. A temperature of 32 ± 1°C was maintained in the system, the application rate was approximately 1.96 mg/cm^2 for Test Preparation 1 and 2.0 mg/cm^2 for Test Preparation 2 (target: 2.0 mg/cm^2). Receptor fluid was collected prior to dosing and 1, 2, 4, 6, 8, 12, 24, 48, and 72 h after dosing. At the last sampling point (72 h), skin samples were washed with an aqueous solution of polysorbate 20 (2%, w/v) and water, dried and removed from the apparatus. The outer layers of the stratum corneum were removed by 5 tape strippings. Epidermis and dermis were then heat separated and all skin samples were analysed by liquid scintillation counting.The limit of reliable measurement was 30 disintegrations per minute (dpm).

Results:

A total of 12 samples of human split skin obtained from 4 different donors were dosed topically with [14C] PHMB in the two cosmetic formulations at a concentration of 0.3% (w/w). In Test Preparation 1 (aqueous micellar solution) and Test Preparation 2 (oil in water emulsion), 53.33% and 58.10%, respectively, were removed during the washing procedure (dislodgeable dose 24 h). At 72 h post dose, the absorbed dose was 0.02% (1.29 ng equiv/cm^2) and 0.03% (1.94 ng equiv/cm^2) of the applied dose, respectively. The “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. The dermis contained 1.23% (82.0 ng equiv/cm^2) and 1.46% (93.4 ng equiv/cm^2) of the applied dose, respectively. The mass balance was complete (92.71% and 99.25% of the applied dose, respectively).

There was negligible increase (0.01% of applied dose) in PHMB concentration observed in the receptor fluid between 24 and 72 h. This confirmed that the PHMB was not migrating through the epidermis/dermis and there was no skin reservoir effect. The results are consistent with those expected for a high molecular weight, polycationic polymer which is highly substantive to negatively charged surfaces such as the skin surface, with the bulk of the material being absorbed onto the stratum corneum which is incompletely removed after only 5 tape strippings.

This observation is relevant to the SCCS Notes of Guidance (2015), in the case of substances with very low dermal absorption and limited permeation (as shown by the respective physico-chemical properties), the epidermis may be excluded when it is demonstrated that no movement from the skin reservoir to the receptor fluid occurs.

Table 6.4.1.2 Skin penetration results for 0.3% [14C]-PHMB in two representative cosmetic formulations with an exposure time of 24 h and a monitoring time of 72 h (expressed as % of applied dose)

Test Preparation	Test Preparation 1	Test Preparation 2
Target [^{14}C]-PHMB Concentration in Test Preparation (%, w/w)	483. 0.30	484. 0.30
485. Actual [^{14}C]-PHMB Concentration in Test Preparation (%, w/w)	486. 0.34	487. 0.32
488. Target Application Rate of Test Preparation (mg/cm^2)	489. 2	490. 2
491. Actual Application Rate of Test Preparation (mg/cm^2)	492. 1.96	493. 2.00
Total Number of Donors	4	4
Total Number of Replicates Dosed	12	12
Total Number of Replicates Contributing to Mean ± SD Data	12	12
494. (% Applied Dose)	495. (Mean ± SD)	496. (Mean ± SD)
497. Dislodgeable Dose 24 h	498. 53.33 ± 7.70	499. 58.10 ± 18.33
500. Total Dislodgeable Dose*	501. 54.23 ± 7.85	502. 59.90 ± 17.47
503. Upper Stratum Corneum (Tapes 1-5)	504. 22.58 ± 3.98	505. 23.24 ± 11.84
506. Unabsorbed Dose **	507. 76.92 ± 7.44	508. 83.31 ± 7.70
509. Epidermis + Lower Layers of Stratum Corneum	510. 14.54 ± 8.18	511. 14.45 ± 6.63
512. Dermis	513. 1.23 ± 0.85	514. 1.46 ± 1.67
515. Receptor Fluid	516. 0.01 ±	517. 0.02 ±

		0.01	0.01
518.	Receptor Chamber Wash	519. <0.01 ± <0.01	520. 0.01 ± 0.01
521.	Absorbed Dose ***	522. 0.02 ± 0.01	523. 0.03 ± 0.02
524.	Mass Balance	525. 92.71 ± 1.64	526. 99.25 ± 5.89

* Total dislodegeable dose = skin wash + tissue swab + pipette tip + donor chamber wash

** Unabsorbed dose = total dislodgeable dose + upper stratum corneum (Tapes 1-5) + unexposed skin

*** Absorbed dose = receptor fluid + receptor wash

Notes

Receptor Chamber Wash = all data was below the Limit of Reliable Measurement (LoRM).

Test Preparation 1 = nine out of twelve samples of 72 h Receptor Fluid was above LoRM. All other samples were below LoRM. Mean and SD were calculated from all samples (n=12) for each time point. Test Preparation 2 = ten out of twelve samples of 72 h Receptor Fluid were above LoRM. All other samples were below LoRM. Mean and SD were calculated from all samples (n=12) for each time point.

Conclusion:

[14C] PHMB in the test preparations was topically applied to human skin *in vitro* at a concentration of 0.3%. Under the present experimental conditions for [14C] PHMB in Test Preparation 1 (aqueous micellar solution) and Test Preparation 2 (oil in water emulsion), 53.33% and 58.10%, respectively, was removed during the washing procedure (dislodgeable dose 24 h). At 72 h post dose, the absorbed dose was 0.02% (1.29 ng equiv/cm^2) and 0.03% (1.94 ng equiv/cm^2) of the applied dose, respectively. The "epidermis + lower layers of stratum corneum" was 14.54% (972 ng equiv/cm^2) and 14.45% (921 ng equiv/cm^2), respectively. The dermis was 1.23% (82.0 ng equiv/cm^2) and 1.46% (93.4 ng equiv/cm^2), respectively. The mass balance was complete (92.71% and 99.25% of the applied dose, respectively). There was negligible increase (0.01% of applied dose) in PHMB concentration in the receptor fluid between 24 and 72 h. This observation is relevant to the SCCS Notes of Guidance (2015), in the case of substances with relevant physicochemical properties including high molecular weight (MW > 500 Da) leading to very low dermal absorption and limited permeation, the epidermis may be excluded when it is demonstrated that no movement from the skin reservoir to the receptor fluid occurs.

Percutaneous absorption study through human skin *in vitro*

Study Design:

Reference: Ref 108, 2014
Date of report: 2014
Guideline/method: OECD 428, OECD Guidance document No. 28, SCCS Basis Criteria (SCCS/1358/10)
Test system: Human skin
Total number

of donors:	7 (33 – 49 years old)
Replicates:	19 (total of dosed), 12 (total used for calculation)
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	a) non-radiolabelled: 09GR24243581, purity: 19.2% aqueous PHMB b) radiolabelled: 99BDR99, [^{14}C]-PHMB, specific activity: 38.9 mCi/g
Purity (radiolabelled):	9.84 % radiochemical purity (HPLC)
Concentration:	0.3% (target, 0.29% actual) in a representative cosmetic formulation
Formulations:	Pre-formulation: P819443, oil/water emulsion without preservative (the composition is provided in the report) Formulation: 819442, formulation containing non radiolabelled test item
Dose level:	2.06 mg/cm²
Exposed area:	3.14 cm²
Skin samples:	Human split skin samples (200 – 400 µm, stored in a freezer at – 20 °C, thawed prior to use) obtained from full-thickness human abdominal skin
Skin temperature:	The skin surface temperature was maintained at 32±1 °C.
Skin integrity:	Tested by electrical resistance barrier integrity test
Test chamber:	Static diffusion cell system
Receptor fluid:	Phosphate buffered saline (PBS).
Route:	topical application
Exposure time:	24 h
Sampling:	pre-dose, 0.5, 1, 2, 4, 8, 24 h
Tape strips:	*Stratum corneum* was removed with 20 successive tape strips
GLP:	Yes
Published:	No

Material and methods:

The percutaneous absorption of radiolabelled [^{14}C]-PHMB (batch: 99BDR99, 38.9 mCi/g) in a representative cosmetic formulation was tested *in vitro* using human split skin samples. The samples were obtained from full-thickness human abdominal skin from 7 donors, processed and stored in a freezer at – 20 °C. After removal from storage, the samples were allowed to thaw at ambient temperature. Split-thickness skin samples were prepared by pinning the full-thickness skin onto a board and cutting to 200 - 400 µm depth using a dermatome. The membranes were laid onto aluminum foil and were stored again in a freezer (-20 °C) prior to use. An electrical resistance barrier integrity test was performed and any human skin sample exhibiting resistance <4 kΩ was excluded from subsequent absorption measurements. The human split skin samples were mounted onto a static diffusion cell system *in vitro* and were topically dosed with [^{14}C]-PHMB in a representative cosmetic formulation (about 0.3%, w/w) at an application rate of 2 mg/cm². The dermal absorption was assessed by collecting receptor fluid aliquots (300 µL, phosphate buffered saline) prior to dosing and at interval of 0.5, 1, 2, 4, 8 and 24 h thereafter. After 24 h of exposure, the skin was washed with an aqueous solution of polysorbate 20 (2%, w/v) and water. The skin was then dried with tissue paper swabs and removed from the cells, dried and the *stratum corneum* removed by 20 tape strippings. PHMB is a highly substantive polymer expected to preferentially and strongly absorb onto the stratum corneum. Residual stratum corneum can be present after up to 20 strippings (Ref 25, 2015) and therefore the amount of PHMB in each tape stripping was quantified to prepare a profile of PHMB concentration in the skin surface layer. The remaining skin was divided into exposed and unexposed skin. The exposed epidermis was separated from the dermis by heat separation. All samples were analyzed by liquid scintillation counting. The limit of reliable measurement was 30 disintegrations per minute (dpm).

Results:

A total of 19 samples of human split skin obtained from 7 different donors were dosed topically with [^{14}C]-PHMB. For the final determination of the dermal absorption, only the results from 12 samples obtained from 5 different donors were considered. The remaining 7 samples have not been taken into account due to processing errors, i.e. samples were dropped on the floor, and due to an inappropriate single high value in the epidermis in one sample being far beyond the other samples.

The mean mass balance was 97.60% of the applied dose at 24 h post dose. At the end of the 24 h exposure period, 49.15% was washed off (5.08%, 41.73%, 2.34% was recovered in the skin wash, tissue swab and pipette tips, respectively). A further 0.54% of the applied dose was removed from the donor chamber wash. The material recovered in the donor wash was almost certainly material that had been dislodged from the skin at 24 h post dose during the washing procedure. Therefore, the total dislodgeable dose was 49.69% of the applied dose. The mean total unabsorbed dose was 94.10% of the applied dose. This consisted of the dislodgeable dose, unexposed skin (0.02%) and the radioactivity associated with the *stratum corneum* (44.40%). The first two tape strips contained 21.84% of the applied dose. There was a steady decrease in the recovery of radioactivity associated with the *stratum corneum*. Tapes 3-5, 6-10, 11-15 and 16-20 contained 11.69%, 7.02%, 2.58% and 1.26%, respectively. Those amounts retained by the *stratum corneum* at 24 h are not considered to be dermally absorbed. The absorbed dose (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%).

The mass balance, dislodgeable dose, unabsorbed dose, absorbed dose and dermal delivery were 5866, 2983, 5658, 10.3 and 208 ng equiv./cm², respectively. Due to the relatively low level of absorption, steady state flux could not be determined.

Table 6.4.1.3 Skin penetration results for 0.3% [14C]-PHMB in a representative cosmetic formulation with an exposure time of 24 h (expressed as ng equiv./cm² and % of applied dose)

Results as percent of applied dose	**Mean ± SD**	**Range**
Total dislodgeable dose (skin wash + tissue swab + pipette tip + donor chamber wash)	49.69 ± 15.64	22.74 – 74.33
Unabsorbed dose (total dislodgeable dose + stratum corneum + unexposed skin)	94.10 ± 5.99	79.83 – 102.4
Absorbed dose (receptor fluid + receptor rinse + receptor wash)	0.17 ± 0.05	0.1 – 0.24
Epidermis	3.18 ± 2.05	0.39 – 6.52
Dermis	0.14 ± 0.10	0.03 – 0.31
Dermal Delivery (epidermis + dermis + absorbed dose)	3.49 ± 2.08	0.66 – 6.95
Mass Balance	97.60 ± 6.38	86.78 – 107.53
Results in ng equiv./cm²	**Mean ± SD**	**Range**
Total dislodgeable dose (skin wash + tissue swab + pipette tip + donor chamber wash)	2982.56 ± 976.05	1403.35 – 4585.98
Unabsorbed dose	5657.61 ± 202.79	5254.77 – 6001.88
(total dislodgeable dose + stratum corneum + unexposed skin)		
Absorbed dose (receptor fluid + receptor rinse + receptor wash)	10.33 ± 3.06	6.05 – 14.56
Epidermis	189.40 ± 120.93	23.84 – 402.51
Dermis	8.21 ± 6.08	1.61 – 20.34
Dermal Delivery (epidermis + dermis + absorbed dose)	207.94 ± 123.77	40.93 – 428.89
Mass balance	5865.55 ± 226.29	5535.71 – 6302.29

* Total dislodegeable dose = skin wash + tissue swab + pipette tip + donor chamber wash
** Unabsorbed dose = total dislodgeable dose + stratum corneum + unexposed skin
*** Absorbed dose = receptor fluid + receptor rinse + receptor wash

**** Dermal delivery = epidermis + dermis + absorbed dose

Conclusion:

[14C] PHMB in the representative cosmetic test preparation was topically applied to human skin in vitro. Under the experimental conditions, 49.69% was removed during the washing procedure (total dislodgeable dose). After 24 h exposure, the absorbed dose (receptor fluid fraction) and dermal delivery (sum of dermis, epidermis, receptor fluid fraction) were 0.17% (10.3 ng equiv/cm^2) and 3.49% (208 ng equiv/cm^2) of the applied dose, respectively. The mass balance was 97.60% of the applied dose and therefore within in the acceptable range.

Human epidermal penetration study *in vitro*

Study Design:

Reference:	Ref 20, 1996
Date of report:	1996
Guideline/method:	Epidermal penetration study *in vitro*
Test system:	Human skin
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	a) non-labelled: no data on batch (20.2% aqueous PHMB solution) b) labelled: [^{14}C]-PHMB on the first and last carbon atom in the monomer, specific activity: 1.85 GBq/732 mg
Dose levels:	nominal: 2, 20, 200 g/L (0.2, 2.0, 20% solutions, analytical: 1.93, 20.9, 197 or 201 g/L)
Volume applied:	10 µL/cm² (non-occluded), 200 µL/cm² (occluded, 200 g/L only)
Exposed area:	2.54 cm²
Skin preparation:	Skin samples were immersed, the epidermis was teased off and the epidermal membranes were stored frozen until use
Replicates:	3 - 8
Skin temperature:	The skin surface temperature was maintained at 30±1 °C.
Test chamber:	Glass diffusion cells
Receptor fluid:	Distilled water
Route:	topical application
Exposure time:	96 h
Sampling:	6, 8, 24, 96 h
GLP:	Yes
Published:	No

Material and methods:

PHMB (as [^{14}C]-PHMB, 20.2% aqueous solution, 1.85 GBq/732 mg) was tested *in vitro* for its percutaneous absorption through human epidermis. The human skin samples were immersed in water at 60^{o}C for 40-45 seconds and the epidermis teased off the dermis. The epidermal membranes were stored frozen on aluminium foil until use. For use, the epidermis samples were defreezed and mounted in glass diffusion cells with an exposed area of 2.54 cm^2. The cells were placed in a water bath maintained at 30±1^{o}C. As the receptor fluid was sampled an equal volume (0.25 ml) of fresh receptor fluid was added to make up the volume lost in sampling. Three concentrations were tested, i.e. 2, 20 and 200 g PHMB/L. The vehicle was water. The PHMB solutions were prepared by mixing unlabelled material with [^{14}C]-PHMB to achieve activities

ranging from about $3x10^6$ dpm/mL for the 0.2% dose up to about $4x10^9$ dpm/mL for the 20% dose to maximise the analytical sensitivity. Each concentration was applied to the test site at a volume of 10 µl/cm^2 without occlusion with the exception that the solution containing 200 g PHMB/L was applied under an occlusive dressing at a volume of 200 µL/cm^2. The exposure period was 96 h and receptor fluid samples were taken at 6, 8, 24 and 96 h of exposure for analysis by Liquid scintillation counting (LCS, limit of quantification (LOQ) between 0.2 – 0.002 µg/mL depending on the application).

Results:

A mass balance recovery determination was not conducted. The absorption of PHMB from the nominal 20% solution through human epidermis was slow and linear over the 96 h exposure period. The permeability coefficient (Kp) has been calculated to be 5.47 x 10^{-7} cm/h from the steady state rate of absorption (0 - 24 h). Approximately 13 times more was absorbed from the 200 µl/cm^2 occluded application (0.088 µg/cm²/h) than from the 10 µl/cm² unoccluded application (0.007 µg/cm²/h).

The absorption rate of PHMB was very slow from the nominal 0.2% solution was measured at 0.001 µg/cm²/h during the first 24 h and was below the limit of quantitation over the 24-96 h period. Absorption from the nominal 2% was 0.005 µg/cm²/h during the first 24 h and slowed down further between 24 and 96h (0.003 µg/cm²/h).

For the 0.2, 2 and 20% solutions from the 10 µg/cm² unoccluded applications, absorption was slow and linear over the initial 24 hours. The steady state rates (0 – 24 h) of absorption are tabulated below:

Table 6.4.1.4 Steady state rates (0 – 24 h) of absorption after unoccluded application of 0.2, 2 and 20% PHMB solutions at a volume of 10 µg/cm²

Dosing Solution (g/L)	**Nominal Dose Applied (µg/cm²)**	**Steady State (0 – 24 h) Rate of Absorption (µg/cm²/h)**
2	527. 20	528. 0.001
529. 20	530. 200	531. 0.005
532. 200	533. 2000	534. 0.009

The details of the absorption expressed as absolute amount (µg/cm²) and percentage absorbed are presented in the table below:

Table 6.4.1.5 Results of the i*n vitro* percutaneous absorption of [^{14}C]-PHMB after exposure of 96 h

PHMB amount	**Time (h)**	**Absolute amount (µg/cm²)**	**Percentage absorbed (%)**
200 µg/cm² (200 g/L, 40,000 µg/cm²) Occluded N = 4	535. 6	536. 0.784	537. 0.001
	538. 8	539. 1.04	540. 0.002
	541. 24#	**542. 2.64**	**543. 0.006**
	544. 96	545. 8.47	546. 0.021

PHMB amount	Time (h)	Absolute amount (µg/cm²)	Percentage absorbed (%)
547. 10 µg/cm² (200 g/L, 2000 µg/cm²) Non-occluded N = 5	548. 6	549. 0.123	550. 0.006
	551. 8	552. 0.141	553. 0.007
	554. 24	**555. 0.237**	**556. 0.012**
	557. 96	558. 0.708	559. 0.036
560. 10 µg/cm² (20 g/L, 200 µg/cm²) Non-occluded N = 8	561. 6	562. 0.023	563. 0.015
	564. 8	565. 0.043	566. 0.020
	567. 24	**568. 0.125**	**569. 0.059**
	570. 96	571. 0.308	572. 0.146
573. 10 µg/cm² (2 g/L, 20 µg/cm²) Non-occluded N = 8	574. 6	575. 0.007	576. 0.036
	577. 8	578. 0.008	579. 0.041
	580. 24	**581. 0.017**	**582. 0.088**
	583. 96	584. 0.025	585. 0.129
586.N = number of samples (replicates) # the figures in bold are the relevant 24 h values with respect to SCCS NOG 2012 requirements			

Conclusion:

Percutaneous absorption of PHMB in aqueous solution through human epidermis *in vitro* was shown to be slow, linear and very low. The non-occluded exposure of 20, 200 and 2000 µg PHMB/cm² for 24 h led to an epidermal absorption of 0.017, 0.125 and 0.237 µg PHMB/cm² (corresponding to 0.088, 0.059 and 0.012%), respectively. Thus, the highest epidermal absorption expressed as percentage value was obtained after exposure to the lowest dose of PHMB.

In addition, the same author performed a dermal penetration study through human epidermis under comparable conditions but at an elevated temperature of 40 °C. PHMB as a 20% solution in water was applied to the epidermal membranes at a rate of 200 µL/cm². One application was occluded for 0.5 h, then the test material washed off and left unoccluded up to 24 h. A separate application was left occluded throughout the entire exposure period of 24 h. These applications were designed to simulate potential human dermal exposure to a 20% solution during use in a Spa bath. The penetration of PHMB applied to human epidermis and washed off after 0.5 h was too low to be measurable at 0.5 h or at 24h (LOQ: 0.05 µg/cm²). The penetration of PHMB applied occluded for 24 h showed a mean absorption rate (0 – 24 h) of 0.118 µg/cm²/h. No penetration was detected at 0.5 h after start of exposure. After 24 h of the start of exposure an amount of 2.84 µg/cm² was absorbed (corresponding to 0.007% of the applied dose).

Table 6.4.1.6 Results of the i*n vitro* percutaneous absorption of [^{14}C]-PHMB after exposure 0.5 or 24 h

PHMB amount	Time (h)	Absolute amount (µg/cm²)	Percentage absorbed (%)
200 µg/cm² (200 g/L, 40,000 µg/cm²) Occluded Exposure: 0.5 h N = 6	587. 0.5	588. <0.05 #	589. <0.0001
	590. 24	591. <0.05 #	592. <0.0001

PHMB amount	Time (h)	Absolute amount (µg/cm²)	Percentage absorbed (%)
593. 200 µg/cm² (200 g/L, 40,000 µg/cm²) Occluded Exposure: 24 h N = 5	594. 0.5	595. <0.05	596. <0.0001
	597. 24	598. 2.84	599. 0.007
600.N = number of samples (replicates) # below LOQ of 0.05 µg/cm²			

Thus, the author concluded that these results indicated negligible dermal absorption at Spa temperatures of 40 °C (Ref 21, 1998).

Prior to the above mentioned studies, an explorative comparative human epidermal and rat skin penetration study *in vitro* was performed with PHMB (as [^{14}C]-PHMB). However, as this study has some limitation with regards to the study design, material and methods, reporting and assessment of the results and especially as a non appropriate method was used to estimate the amount of penetration, no reliable quantitative information can be derived from this study. This study is only presented for sake of completeness. A series of experiments was performed covering absorption of [^{14}C]-PHMB across human epidermis and across rat whole skin-measured, absorption followed after application of a small volume, which was allowed to dry under ambient conditions and uptake of [^{14}C]-PHMB by human epidermis assayed after equilibration with a wide range of PHMB concentrations (0.001% - 20%).

It was noted that when applied and maintained as aqueous solutions, [^{14}C]-PHMB absorption through human epidermis was slow and variable but approximately proportional to concentration. The obtained results indicated qualitatively that for rat whole skin, the mean absorption rate of [^{14}C]-PHMB from aqueous solution was about 16 times faster than through human epidermis and from 20% PHMB was about 3 and 12-fold faster than human. Thus, under the conditions of the study it can generally be concluded that absorption of PHMB through intact human epidermis was very slow and that rates of absorption of PHMB through rat whole skin were faster (Ref 31, 1982).

There exists also a previously performed comparative penetration study of lower reliability. Radiolabelled PHMB ([C^{14}]-Vantocil, no further information) was applied to skin biopsies of newborn, hairless rats. The skin biopsy samples were punched and placed in chamber filled with medium on a perforated plate of stainless steel placed on filter paper. PHMB was applied as 5% solution and samples were taken during the first 12 hours at one hour intervals and thereafter 3 samples were taken daily during a period of 8 hours for the following 4 consecutive days. In comparison, four biopsies of human skin were punched, the epidermis was separated and treated as in the part with rat skin biopsies with the exception that after 48 hours, 1 ml DMSO was added. The radioactivity was measured by means of liquid scintillation counter (LKB-Wallace 81000). In the study part using rat skin biopsies, no skin absorption was detected up to day 5 of exposure. In contrast, in human epidermal skin biopsies a low rate of penetration of about 0.09% was noted after 24 hours and this penetration rate was from 0.11% up to 0.81% after adding DMSO (Ref 69, 1976a).

6.4.3 Overall discussion and conclusion on dermal/percutaneous absorption

Taken together, the experimental results from all available *in vitro* skin penetration studies are consistent with those expected by theoretical considerations and confirm that PHMB is a very poor skin penetrant (Refv 49, 2016).

In Submission I, the available *in vitro* studies on percutaneous penetration of PHMB were not deemed in compliance with SCCS requirements. Therefore for Submission II an additional dermal penetration study was performed with radiolabelled PHMB at 0.3% in a representative cosmetic formulation (oil in water emulsion, o/w) according to OECD TG 428 and SCCS basic requirements for dermal penetration studies and in line with GLP principles (Ref 108, 2014). A total of 20 tape strippings was performed and the PHMB present in each tape stripping was quantified to produce a profile of the absorbed PHMB through the stratum corneum/upper epidermis. The SCCS considered the study acceptable, but decided to use the mean of the dermally delivered dose plus 2 SD, i.e. 3.49 + 4.16 % = 7.65 % of the applied dose, as skin absorption rate for the calculation of the Systemic Exposure Dose (SED). This decision was particularly based on the number of tape strippings used to remove stratum corneum which was deemed unusually high (n=20), the fact that dermal penetration was only investigated in one type of cosmetic formulation (o/w emulsion) and the variability observed in the study.

In order to respond to the key comments of the SCCS, and also to evaluate whether the amounts of PHMB found in the stratum corneum and epidermis are available for systemic exposure, two new *in vitro* dermal penetration studies were performed, one with 0.3% PHMB (current maximum concentration permitted according to Annex V of the Cosmetics Regulation, previously tested concentration) and one with 0.1% PHMB. Both were conducted with two different representative cosmetic formulations, one which facilitates penetration (o/w emulsion) and one which is the most representative of products on the market (aqueous micellar solution), using a methodology consistent with both the OECD and the SCCS requirements (Ref 109, 110, 2016ab).

The objective of the first new study was to determine whether the amount of PHMB found in skin compartments of the previous 24 h standard study with 0.3% PHMB in o/w emulsion represented a genuine skin reservoir for potential systemic exposure or whether these residues are systemically unavailable and, therefore, can be reasonably excluded. Thus, the study design was based on a refined test protocol including a standard 24 h exposure to 0.3% PHMB in two different representative cosmetic formulations (aqueous micellar solution, o/w emulsion), measurement of penetration into receptor fluid for up to 72 h and determination of PHMB remaining in the stratum corneum, epidermis, and dermis at 72 h.

The second new study assessed the *in vitro* skin penetration of 0.1% PHMB in two different representative cosmetic formulations (aqueous micellar solution, o/w emulsion) according strictly to the SCCS and OECD guidelines with the usual exposure and monitoring time of 24 h in order to provide the skin penetration rate for the PHMB use concentration of 0.1%. No measurement was performed at 72 h as evidence was obtained in the previous study at 0.3% that there was no reservoir effect.

In both new studies, only 5 tape strips of the stratum corneum were taken which did not remove the entire stratum corneum. As a result, the quantity of radioactivity recovered in the next sampled layer included that in the epidermis as well as that in the residual stratum corneum that had not been tape-stripped. Comparison with results from the previous 24-h study with 0.3% PHMB – in which most of the stratum corneum was removed by taking 20 tape strips – reveals

that the percentage of applied radioactivity in the first 5 tapes from the former study are indistinguishable from those measured in the recent ones (with little difference depending on formulation, concentration or monitoring time). Therefore, the sum of the quantities in tape strips 6-20 and that in the epidermis from the first study (~14.1%) is essentially identical to the quantities attributed to the "residual stratum corneum + epidermis" layer in the new studies. Thus, it can be reasonably considered that a substantial fraction of PHMB in the "residual stratum corneum + epidermis" is, in fact, in the residual stratum corneum. Furthermore, the fact that the percentage of radioactivity in this compartment did not change significantly when the monitoring time was increased from 24 to 72 hours implies that the compound present in this tissue was not mobile and did not migrate further into the skin (Ref 49, 2016).

Overall, mass balance of the two new studies was excellent, *i.e.* 91-99%. At least 50% of the applied radioactivity did not enter the skin at all, similar to the previous skin penetration study at 0.3% PHMB. There was no relevant difference between the disposition of the radioactivity either as a function of formulation or as a function of monitoring time. With both formulations (aqueous micellar solution, o/w emulsion), the quantities reaching the receptor compartment of the *in vitro* diffusion cell were between 0.02 and 0.04% of the applied amount. Moreover, a predominant proportion of the receptor phase samples contained levels of radioactivity below the quantifiable limit of 30 dpm above background. Both with 0.3% and with 0.1% PHMB formulations, the majority of the supposedly absorbed dose (85-95%) was detected in the "residual stratum corneum + epidermis" fraction indicating that PHMB does not penetrate further. The average level of the applied dose recovered from the dermis was consistently about 1%, with substantial variability (Ref 49, 2016).

Given the aqueous solubility of PHMB, and the relatively similar, "aqueous gel-like" nature of the viable epidermis and dermis, a ready diffusion from epidermis to dermis may be reasonably expected. The fact that this movement did not occur over a 48-h period (i.e., the time interval between 24 and 72 h) further supports the 'non-mobility' of most, if not all, of the PHMB recovered from the "residual stratum corneum + epidermis" layer. In conclusion, the kinetics of skin penetration over the 72-h monitoring period demonstrated that the PHMB content of the "residual stratum corneum + epidermis" reflects only the absorption of PHMB to the residual stratum corneum and that the PHMB fraction present in the "residual stratum corneum + epidermis" does not act as a reservoir for continued percutaneous penetration. Based on this, there is no evidence that any radiolabelled PHMB recovered from the "residual stratum corneum + epidermis" layer would ever contribute significantly to the body burden of PHMB. Thus, the amounts in the epidermis can be reasonably excluded from the calculation of the systemic exposure dose (Ref 49, 2016).

For the MoS calculation, the skin penetration rate of the aqueous micellar solution containing 0.1% PHMB was taken as it is the representative formulation for a large range of cosmetic products using PHMB, and it is higher than with the o/w emulsion formulation representing a more conservative approach. Since the study was fully compliant with the requested guidelines and incorporated more than one type of cosmetic formulations, only 1 SD was added as required by the SCCS Notes of Guidance, resulting in a skin penetration rate of 4.09% (1.56% in dermis + 0.03% total absorbed + 2.5% *i.e.* 1 SD) in aqueous micellar solution.

6.5 Repeated dose toxicity

6.5.1 Subacute toxicity

6.5.1.1 Subacute oral toxicity

Study Design:

Reference:	Ref 55, 1992a
Date of report:	1992
Guideline/method:	Range-finding study with few parameters in clinical chemistry and no histopathology
Species/strain:	Rat/Sprague-Dawley
Group size:	8 male and 8 female rats per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	D4097 (Vantocil P, 20.2% aqueous PHMB solution)
Vehicle:	Water
Dose levels:	0, 0.1, 0.5, 1.0, 2.0 mg/mL (corresponding to about 0, 16, 77, 151, 302 mg/kg bw/day in males; 0, 18, 76, 156, 325 mg/kg bw/day in females)
Route:	Oral (drinking water)
Exposure period:	28 days
Exposure frequency:	daily
GLP:	Yes
Published:	No

Material and methods:

The subacute oral toxicity of PHMB (Vantocil P, batch: D4097, 20.2% aqueous PHMB solution) was examined in a range-finding study in male and female Sprague-Dawley rats after repeated oral intake via the drinking water for 28 consecutive days. Each 8 male and 8 female rats received PHMB in their drinking water at concentrations of 0, 0.1, 0.5, 1.0 or 2.0 mg/mL. The stability of PHMB in the drinking water was analysed prior to the start of the study. Clinical observations were recorded daily throughout the study and body weight was recorded immediately prior to administration of the experimental drinking water on day 1, daily for the first week of the study and weekly thereafter. Food consumption for each cage of rats was recorded daily for the first week of the study and weekly thereafter. Water consumption for each cage of rats was recorded daily throughout the study. At termination of treatment, all surviving animals were killed and subjected to a gross post mortem examination, and liver and kidney weights were recorded. A sample of blood was taken from for analysis of plasma urea, creatinine, albumin, total protein and cholesterol levels, and plasma alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) activities. Histopathological examination of the tissues from this study was not performed.

Results:

Analysis of the dosing preparation showed stability during a period of 7 days and confirmed the correctness of the concentration.

One male and one female were killed due to severe body weight loss and deterioration of clinical conditions at 2.0 mg/mL. All other animals of this and lower dose groups survived until termination. Loss in body weight was recorded for the majority of animals receiving 1.0 or 2.0 mg/mL, and for several animals at 0.5 mg/mL, over the first 1 to 3 days of treatment, with a

subsequent recovery. Mean body weight and body weight gain for animals receiving 2.0 mg/mL was statistically significantly reduced. Food consumption was slightly reduced at 1.0 or 2.0 mg/mL during the first 1 to 3 days of treatment. From days 3 to 4 of treatment, food intake improved for these animals. Water consumption was reduced for males from all treated groups, and for females at 0.5, 1.0 or 2.0 mg/mL, during the first 1 to 2 days of treatment. Increased water intake was noted for these animals from day 2 to 3, although group mean water intake for males and females at 1.0 or 2.0 mg/mL, and for females at 0.5 mg/ml, was often slightly lower than that recorded for control animals throughout the remainder of the study. At terminal kill group mean plasma cholesterol levels and alkaline phosphatase activities for males and females receiving 2.0 mg/mL, and group mean plasma aspartate transaminase activity for males, were statistically significantly increased. At post mortem examination there were no macroscopic findings. Mean relative kidney weights from all treated groups were dose-dependently and statistically significantly increased. Mean relative liver weights at 1.0 mg/ml were increased with the value for males attaining statistical significance. Mean relative liver weight at 2.0 mg/mL was lower than that of control.

Conclusion:

The subacute administration of PHMB via the drinking water for 28 days at 0, 0.1, 0.5, .10, 2.0 mg/mL (corresponding to about 0, 16, 77, 151, 302 mg/kg bw/day in males; 0, 18, 76, 156, 325 mg/kg bw/day in females) led to a dose-related loss in body weight and/or reduced weight gain and reduced water and/or food consumption at dose levels of 0.5, 1.0 and/or 2.0 mg/mL. These effects occurred predominantly during the first few days of treatment. The treatment led to increases in plasma cholesterol level and ALP and AST activities at 2.0 mg/mL, increased liver weight at 1.0 mg/mL, with a decrease in liver weight at 2.0 mg/mL, and a dosage-related increase in kidney weight for all treated groups. A No Observed Effect Level (NOEL) was not achieved in this study. However, as no histopathology was performed, the relevance of the elevated kidney and liver weights remained uncertain and can therefore finally not considered as adverse effects. In addition, this study indicated clearly a decrease in palatability of the selected PHMB dose levels, when applied via the drinking water. Therefore, this route of application can be considered as not appropriate for long-term application in rats.

A comparable subacute range-finding study with PHMB (Vantocil P, batch: D4097, 20.2% aqueous PHMB solution) was carried out in C57Bl mice. Each 10 male and 10 female animals received PHMB in their drinking water at concentrations of 0, 0.1, 0.3, 0.6 and 1.2 mg/mL (corresponding to about 0, 21, 49, 84, 146 mg/kg bw/day in males; 0, 21, 51, 99, 175 mg/kg bw/day in females) for 28 days. This application procedure led to a dose-related initial loss of body weight and reduction in food and water consumption, followed by a reduction in body weight gain and/or continued reduction in water consumption, for males treated with 0.1, 0.3, 0.6 or 1.2 mg/mL and females treated with 0.3, 0.6 or 1.2 mg/mL. An initial body weight loss only was noted for females treated with 0.1 mg/mL. These initial changes were considered to reflect reduced palatability of PHMB. Other changes included a decrease in plasma alanine transaminase activity and a decrease in liver weight for males at 0.6 or 1.2 mg/mL. A NOEL was not achieved in this study. However, as no histopathology was performed, the relevance of the observed findings remained. In addition and as already observed in rats, this study indicated also a clear decrease in palatability of the selected PHMB dose levels, when applied via the drinking water. This could have led to secondary effects. Therefore, this route of application can be considered as not appropriate for long-term application in mice (Ref 56, 1992b).

Study Design:

Reference: Ref 95, 1993
Date of report: 1993

Guideline/method:	Range-finding study
Species/strain:	Dog/Beagle
Group size:	1 male and 1 female dog per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	no data (20% aqueous PHMB solution)
Dose levels:	0, 2500, 4000, 5500 ppm (corresponding to about 0, 90, 150, 200 mg/kg bw/day)
Route:	Oral (diet)
Exposure period:	6 weeks
Exposure frequency:	daily
GLP:	Yes
Published:	No

Material and methods:

The subacute oral toxicity of PHMB (20% aqueous PHMB solution) was examined in a range-finding study in male and female Beagle dogs after repeated oral intake via the diet for 6 weeks. Each 1 male and 1 female dogs received PHMB at dietary concentrations of 0, 2500, 4000 and 5000 ppm (corresponding to about 0, 90, 150, 200 mg/kg bw/day). After preparation, the pelleted diet was stored at room temperature. In the absence of a reliable method for quantitative analysis for PHMB in diet, the measurement of achieved concentration, homogeneity and chemical stability of PHMB in diet was not determined. All dogs were detailed clinically examined at pre-study and at termination. During the study the dogs were observed frequently for gross clinical or behavioural abnormalities. Dogs were weighed weekly pre-study and during treatment, and the amount of food consumed by each dog was recorded. A record of faecal consistency was made daily for two weeks pre-study and then throughout the study. Haematology and clinical chemistry examinations were performed on blood taken from all dogs, once pre-experimentally and then during weeks one, three and six. At the end of the treatment period, the dogs were anaesthetised by an intravenous injection of sodium pentobarbitone and killed by exsanguination. A full post mortem examination was carried out an all dogs. The liver, testes, epididymides, brain, thyroid with parathyroid and kidneys were weighed. These organs and any other organ with gross findings were submitted for histological processing and microscopic examination. A bone marrow smear was made from a rib of each dog.

Results:

There was no mortality in any group. The female dog fed 5500 ppm was noted as thin towards the end of the study. There were no treatment-related clinical signs in the males at any dose level. The female dog at the highest dose level lost weight throughout the study and showed a reduction in food consumption of approximately 50% from days 3 to 43. There was no effect on body weight gain or food consumption for the male at this dose level or for either sex at the lower dose levels. There were no hematological changes related to the administration of PHMB at any dose level. At 5500 ppm there were reductions in plasma cholesterol in both sexes throughout the study. Plasma alanine and aspartate transaminase activities were raised from week 3 in the female and week 6 for the male fed 5500 ppm. The female at this dose level also showed an increase in creatine kinase activity throughout the study and plasma alkaline phosphatase activity was reduced by the end of the study. At 4000 ppm, plasma alanine transaminase activities were raised in both sexes at week 6 and in the female only at week 3. The plasma creatine kinase activity of the female at this dose level was also slightly raised in weeks 3 and 6. There were no effects at 2500 ppm. At necropsy, the absolute liver weight and the absolute and relative testes weights of the male dog at 5500 ppm were reduced. The histopathological examination revealed treatment related findings confined to the liver and

kidney at 4000 and 5500 ppm. Both dogs fed 5500 ppm showed slight/moderate hepatocellular intracytoplasmic eosinophilic inclusion bodies, minimal/slight hepatocellular single cell necrosis and moderate focal hepatocellular swelling. The male also showed minimal hepatocellular proliferation. The female showed slight focal macrophage/Kupffer cell pigmentation in the liver. The male showed slight hyaline droplet formation in the renal cortical tubules. Both dogs fed 4000 ppm showed minimal/slight hepatocellular intracytoplasmic eosinophilic inclusion bodies. The female showed slight hyaline droplet formation in the renal cortical tubules. Ultrastructural evaluation of the liver and kidney in the male at 5500 ppm showed megamitochondria in the cells of the centrilobular region of the liver and confirmed the presence of hyaline droplets in the renal cortex.

Conclusion:

The subacute administration of PHMB via the diet for 6 weeks to one male and one female dog at dose levels of 0, 2500, 4000 and 5500 ppm showed signs of systemic toxicity at both high dose levels. At 5500 ppm reduced food intake and weight loss in the female was observed. At 4000 and 5500 ppm there was evidence of liver toxicity characterised by reductions in plasma cholesterol, increases in the activities of specific plasma enzymes associated with liver changes and microscopic hepatocellular changes. There was also evidence for minor kidney changes at these dose levels. The NOEL for male and female dogs was 2500 ppm (corresponding to about 90 mg/kg bw/day).

Previously to the above study, a maximum tolerated dose study was performed in male and female Beagle dogs. PHMB (20% aqueous PHMB solution) was administered orally, either by gelatine capsule, by gavage or in diet, usually immediately prior to feeding. All dogs were detailed clinically examined at pre-study and at termination. During the study dogs were observed for gross clinical or behavioural abnormalities, were weighed weekly pre-study and during treatment, and the amount of food consumed by each dog was recorded. A record of faecal consistency was made daily for two weeks pre-study and then throughout the study. At the end of the treatment period the dogs were anaesthetised by an intravenous injection of sodium pentobarbitone and killed by exsanguination. The dogs were macroscopically inspected and any abnormalities were submitted for histological processing and microscopic examination. Initially the study consisted of one group, comprising one male and one female dog, receiving 100 mg/kg bw/day by capsule gavage in water or corn oil or gavage in milk. Both methods of administration (capsule or gavage) at 100 mg/kg bw/day resulted in vomiting/regurgitation within 30 minutes of dosing. The dose level was decreased to 25 mg/kg bw/day and administered by gavage for 1 week. Vomiting or regurgitation was still seen in the male, but not in the female. The dose level was increased on a weekly basis as follows: 50, 75 and finally 100 mg/kg bw/day. The male continued to vomit and regurgitate and was terminated after one dose of 75 mg/kg bw/day. The weight gain was unaffected but post mortem examination revealed diarrhoea and a fluid filled gastrointestinal (GI) tract. Both dogs salivated and showed resistance to dosing by gavage. The female showed no weight gain at 75mg/kg/day and vomited/regurgitated and in addition had fluid faeces, whilst dosed at 100 mg/kg bw/day. During the last week of dosing the female showed a body weight loss and post mortem examination revealed slightly enlarged mesenteric lymph nodes. A further one male and one female dog were started on study at 25 mg/kg bw/day, administered in gelatine capsules as neat PHMB (20% a.i.) for one week. Both dogs initially vomited or regurgitated but then tolerated the dose. The dose level was increased to 50 mg/kg bw/day for 3 weeks. Both dogs had vomited/regurgitated about 50% of the doses. Body weight gain was no affected in this group. At post mortem, the male had diarrhoea, a fluid filled gastrointestinal tract and dark areas on the stomach lining. The female had fluid contents of the upper GI tract only. Histopathological examination of the male stomach revealed only surface

debris and exfoliated cells, indicative of a low grade catarrhal gastroenteritis. A third group comprising one male and one female dog was started on study with PHMB administered in pelleted diet at 4500 ppm for 9 days. However, heating during pelleting affected PHMB and therefore, the dogs were transferred to control powdered diet for 5 days and then fed 4500 ppm in powdered diet for 9 days. In the absence of an effect on body weight gain after 9 days, the dose level was increased to 7000 ppm in pelleted diet. This resulted in severe emesis within 24 hours, body weight loss and both dogs were terminated on the second day at 7000 ppm. Post mortem examination revealed abnormal contents in the GI tract of both dogs.

In summary PHMB, when administered orally by capsule or gavage, resulted in emesis and irritation of the gut at all dose levels tested. The dietary route of administration was tolerated better and will be used in subsequent studies in the dog. A dietary dose level of 7000 was clearly in excess of a maximum tolerated dose. No adverse effects were observed during this maximum tolerated dose study at 4500 ppm (Ref 94, 1992).

6.5.1.2 Subacute dermal toxicity

Study Design:

Reference:	Ref 76, 1993
Date of report:	1993
Guideline/method:	Comparable to OECD 410
Species/strain:	Rat/Alpk:APfSD (Wistar derived)
Group size:	5 male and 5 female rats per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	no data on batch (Vantocil P, 20.2% aqueous PHMB solution)
Dose levels:	0, 20, 60, 200 mg/kg bw/day
Route:	Dermal (occlusive)
Study period:	30 days
Exposure frequency:	5 applications/week
Exposure duration:	6 h/day
Total applications:	21
GLP:	Yes
Published:	No

Material and methods:

The subacute dermal toxicity of PHMB (Vantocil P, 20.2% aqueous PHMB solution) was examined in Alpk:APfSD rats. Groups of five male and five female rats received six-hour dermal applications to the previously shorn back at dose levels of 0, 20, 60 and 200 mg/kg bw/day under an occlusive dressing during a period of 30 days. The selected dose levels were based on the result of a preceding range-finding study. The dose of 200 mg/kg bw/day presented the highest dose level considered unlikely to cause a level of skin irritation, which could preclude repeated dermal application of PHMB. The animals received a total of 21 applications (five applications per week). There were four two-day periods when the animals were not dosed. Following each application there was an 18-hour rest period during which the animals were fitted with plastic collars to prevent oral contamination. The animals were not fitted with collars on the days they were not dosed. At termination of treatment, all rats were bled by cardiac puncture and samples for haematology and clinical pathology were taken and analysed. Thereafter, all the animals were killed by exsanguination under terminal anaesthesia induced by halothane and were subjected to gross pathology. The testes, kidneys and liver were weighed adrenal, brain, kidney, liver, treated skin, untreated skin, testes, epididymides and any macroscopically abnormal tissue were

processed and preserved for histopathological examination. Statistical analyses were performed by means of an analysis of variance (ANOVA) and the two-sided Student's test

Results:

There were no mortality and no overt clinical signs of toxicity at any dose-level. There were no substance-related effects on body weight, food consumption, organ weights, haematology or clinical chemistry. Gross pathology and histopathology revealed no evidence of systemic toxicity at any dose level. Signs of skin irritation were dose-dependently noted at 60 or 200 mg/kg bw/day. At 60 mg/kg bw/day there was slight irritation, which in most animals had regressed by the end of the study. At 200 mg/kg bw/day all the animals showed moderate irritation, which in most animals persisted until the end of the study. The irritation noted during the study was confirmed microscopically and was indicative of a compound-related effect.

Conclusion:

The subacute dermal administration of PHMB under an occlusive dressing at dose levels of 0, 20, 60 and 200 mg/kg bw/day for 6 h/day during a 30 day period (21 applications in total) led to no sign of systemic toxicity in male and female rats. Signs of slight to moderate skin irritation occurred dose-dependently increased in severity and duration at 60 and 200 mg/kg bw/day.

The NOAEL for systemic toxicity was 200 mg/kg bw/day, the highest dose level investigated.

The NOAEL for local dermal irritation was 20 mg/kg bw/day.

In principle, the above results are in line with a previously performed study with lower reliability. PHMB (Vantocil IB) was applied to a group of six female albino rabbits daily to their shorn backs for 23 hours in the form of 1.0 ml of a 12,000 ppm solution. The skin was then washed with soap and water and the solution was re-applied one hour later for a total of 21 daily applications. The skin was not occluded and oral contamination was prevented by means of a plastic collar. At the end of the experiment the rabbits were killed. Blood was taken for haematological examination and liver, adrenal, kidney, spleen, ovary, uterus, heart, thymus and lung were examined histopathologically. No signs of systemic toxicity or skin irritation were noted during the experimental period and the animals gained body weight at the same rate as control animals. There were no signs of organ damage at autopsy and histopathological examination of the tissues did not reveal any changes attributable to treatment. Haematological findings indicated no significant differences between test and control animals (reference: Ref 36, 1972).

6.5.1.3 Subacute inhalation toxicity

Study Design:

Reference:	Ref 91, 2006c
Date of report:	2006
Guideline/method:	OECD 412, Council Directive 67/548/EEC, OPPTS 870.3465
Species/strain:	Rat/Alpk:APfSD (Wistar derived)
Group size:	5 male and 5 female rats per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	Bx6142 (Vantocil IB, 19.6% aqueous PHMB solution)
Concentrations:	a) main study: 0, 0.025, 0.25, 2.5 mg/m³ b) recovery study: 0, 0.025, 2.5 mg/m³
Route:	Inhalation (nose only)
Exposure:	6 h/day, 5 days/week for 28 days
Recovery:	13 week (control, low, high concentration)
GLP:	Yes

Published: No

Material and methods:

The subacute inhalation toxicity of PHMB (Vantocil IB, batch: Bx6142, 19.6% aqueous PHMB solution) was examined in Alpk:APfSD rats. Groups of five male and five female animals were exposed nose only for 6 hours per day to target concentrations of 0 (control), 0.025, 0.25 or 2.5 mg/m³ for 6 hours per day, 5 days per week, over a period of 28 days (main study). Additional groups of five animals, of both sexes were similarly exposed to 0, 0.025 or 2.5 mg/m³ and then retained without treatment for a further 13 weeks (recovery study). Clinical observations were made twice daily on exposure days, once daily on non-exposure days and then weekly during the recovery period. Bodyweights were measured weekly and food consumption was measured continuously throughout the study. At the end of the scheduled period, the animals were killed and examined by gross pathology. Cardiac blood samples were taken for haematology and clinical pathology from all animals. The adrenal glands, ovaries, epididymides, spleen, kidneys, testes, liver, thymus, lungs (with trachea attached but larynx removed), brain, heart, uterus and cervix were weighed. Specified tissues were taken and processed for subsequent histopathology examination. Statistical analyses were performed by means of an analysis of variance (ANOVA) and the two-sided Student's test

Results:

The analysed concentrations of PHMB were 0.0239 mg/m³ (MMAD range – 0.32-1.30 μm), 0.257 mg/m³ (MMAD range – 0.48-5.06 μm) and 2.47 mg/m³ (MMAD range – 0.67-1.67 μm) for the low, mid, and high dose group, respectively. There were no deaths attributable to treatment. There were no clinical signs that were attributable to exposure to PHMB at up to 2.5 mg/m³. Clinical observations during the exposure periods and post-exposure were typical of those associated with the restraint of the animals for a nose-only exposure. Body weights were lower than for the controls for males exposed to 0.25 mg/m³ or 2.5 mg/m³. There was some evidence of recovery in body weight, following cessation of exposure for males at 2.5 mg/m³. Food consumption was slightly low in weeks 2 and 4 for males exposed to 0.25 and 2.5 mg/m³. There were no changes in haematology or blood clinical chemistry parameters that were of toxicological significance. Lung weights were slightly high for males and females exposed to 2.5 mg/m³ and thymus weights slightly high for males only at this exposure concentration. No macroscopic treatment-related findings were observed at the examination post mortem. Treatment-related microscopic findings were recorded in the larynx, trachea and lungs. On completion of the 28 day exposure period, squamous metaplasia was seen in the larynx of males and females at 0.25 and 2.5 mg/m³, and tracheal inflammation for males and females at 2.5 mg/m³. No similar findings were present 13 weeks following cessation of treatment for animals previously exposed to 2.5 mg/m³. Pneumonitis and bronchitis in the lung were seen for males and females exposed to 2.5 mg/m³, both at end of the exposure period and at the end of the recovery period. However, the pneumonitis was observed to be slightly reduced in severity at the end of the recovery period. Since the pneumonitis and bronchitis were only observed at the high concentration, it is judged to be the result of a primary irritant response. The higher thymus weight for males only exposed to 2.5 mg/m³, in the absence of any histopathological changes, was considered to be of unknown toxicological significance.

Conclusion:

The subacute inhalation of PHMB at concentrations of 0, 0.025, 0.25 or 2.5 mg/m³ (nominal) for 6 hours per day, 5 days per week, over a period of 28 days led to substance-induced findings at 0.25 and 2.5 mg/m³ in the form of some transient histopathological changes in the larynx and trachea that were characteristic of exposure to a respiratory tract irritant. These changes were

clearly reversible following cessation of exposure during the recovery period. Body weight changes were also present at these exposure concentrations. Some non-resolving histopathology changes in the lungs (pneumonitis and bronchitis) were limited to the highest exposure concentration of 2.5 mg/m³. The NOAEL for systemic toxicity was 0.025 mg/m³ in male and female rats under the condition of the study.

Previously to the above study, two range-finding studies were performed under GLP conditions. In the first study, groups of two male and two female Alpk:APfSD rats were exposed to PHMB as 19.6% aqueous solution (Vantocil IB, batch: Bx6142) to target concentrations of 0 (control), 0.050, 0.125, 0.5 and 5.0 mg/m³ (corresponding to 0, 0.01, 0.025, 0.1 and 1.0 mg PHMB/m³) for 6 hours per day over a period of 5 consecutive days. Clinical observations, body weights and food consumption were recorded daily throughout the study and at the end of the scheduled period, the animals were killed and examined *post mortem*. Selected organs were weighed and specified tissues were taken, with a limited tissue list (lung and larynx) for subsequent histopathology examination. The analysed test atmospheres were close to the target concentrations and were considered acceptable with regard to their general stability during exposure periods and physical characteristics. The analysed concentrations of PHMB were 0.0093 mg/m³ (MMAD range: 2.20-4.05 µm), 0.0371 mg/m³ (MMAD range: 2.46-3.82 µm), 0,126 mg/m³ (MMAD: range 1.14-2.27µm) and 1.12 mg/m³ (MMAD range: 1.44-2.43 µm) for the target concentrations of 0.01, 0.025, 0.1 and 1.0, respectively. There were no deaths and no clinical signs attributable to exposure to up to analysed 1.12 mg PHMB/m³. Clinical observations during the exposure periods and post-exposure were typical of those associated with the restraint of the animals for a nose only exposure. The body weight gains were marginally lower than for the controls for males and females exposed to 1.12 mg/m³ and food consumption minimally low for males only at this exposure concentration. There were no clear exposure related effects on organ weights or gross pathology. Exposure related histopathological findings were confined to the larynx and were characterised by minimal squamous epithelial metaplasia in the anterior wall for all males exposed to 0.126 or 1.12 mg/m³, and one out of two males exposed to 0.0371 mg/m³. Thus, the nose-only exposure for 6 hours per day for a period of 5 consecutive days to PHMB at resulted in some histopathological changes in the larynx characteristic of exposure to a respiratory tract irritant (Ref 89, 2006a).

The second range-finding study was performed under identical conditions with the exception that two rats per sex and group were exposed to PHMB target concentrations of 0, 5 and 15 mg/m³ (analysed: 5.61, MMAD range: 0.91-2.12 µm and 14.0 mg/m³, MMAD range: 1.54-2.62 µm). There were no deaths. The clinical signs noted at both concentrations were indicative of possible respiratory tract irritation. An increased response to touch and hunched posture were also noted for females at 14.0 mg/m³. Initially the animals lost body weight but body weight gained overall during the study, with the exception of the males at the high concentration. The food consumption was also reduced. There was a slight increase in lung weights in males exposed to 5.61 mg/m³ and females at 14.0 mg/m³. Mottled and pale lungs were observed at the examination *post mortem*. Histopathology confirmed concentration-dependently increased findings in the lung and larynx indicative of respiratory tract irritation (Ref 90, 2006b).

There exists also an older 28-day inhalation study with lower reliability. In this study groups of four rats were exposed to atmospheres containing respirable particles of PHMB (Vantocil IB, 20% aqueous PHMB solution) at concentrations of 0.025, 0.25, 2.75, 12.5 and 26 mg/m³ for 6 hours per day for 5 days a week, for three weeks, snout-only. The study was performed before adoption of guidelines and its interpretation was limited by poor reporting. In the high dose group of 26 mg/m³, very severe nasal irritation and marked dyspnoea were noted ante-mortem, only a single exposure was possible and all treated rats died within 24 hours of first exposure.

The concentration of 12.5 mg/m³ respirable particles proved particularly toxic. Severe nasal irritation and dyspnoea were evident and all rats died following the fourth exposure period. At lower concentrations, 2.75 or 0.25 mg/m³, moderate to severe eye and nasal irritation was seen with associated pneumonitis; body weight gains, food and water intakes were all reduced and methemoglobin was evident. Mortality was also observed at 2.75 mg/m³ in 4 animals during or after the sixth exposure. The thymus glands from all PHMB exposed animals at 2.75 mg/m³ showed severe depletion of lymphocytes and loss of normal architecture. Haematological examination revealed haemoconcentration and significant increases of methemoglobin for all animals exposed to 2.75 or 0.25 mg/m³. At 0.25 mg/m³, one male died after the 13th exposure. The low concentration of 0.025 mg/m³ did not result in any sign signs of toxicity but the body weight gain was low and erratic. The NOAEL under the conditions of the study was therefore 0.025 mg/m³ (Ref 16, 1976).

6.5.2 Subchronic toxicity

6.5.2.1 Subchronic oral toxicity

90-day dietary sighting study in rats:

Study Design:

Reference:	Ref 58, 1993a
Date of report:	1993
Guideline/method:	Range-finding study with an extent comparable to OECD 408 but fewer parameters in haematology, clinical pathology and pathology
Species/strain:	Rat/Alpk:APfSD (Wistar derived)
Group size:	12 male and 12 female animals per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	D4097 (Vantocil P, 20.2% aqueous PHMB solution)
Dose levels:	0, 1000, 2000, 4000, 6000 ppm (corresponding to about 0, 83.9, 171.5, 373.0, 556.1 mg/kg bw/day in males, 0, 92.3, 192.9, 409.8, 617.4 mg/kg bw/day in females)
Route:	Oral (diet)
Exposure period:	90-days (terminal kill, 8 animals per sex and group)) 29-days (interim kill, 4 animals per sex and group)
Exposure frequency:	daily
GLP:	Yes
Published:	No

Material and methods:

The subchronic toxicity of PHMB (Vantocil P, batch: D4097, 20.2% aqueous PHMB solution) was examined within a sighting study for a subsequent long-term feeding study in Alpk:APfSD (Wistar derived) rats. Groups of 12 male and 12 female rats were fed diets containing PHMB at dose levels of 1000, 2000, 4000 or 6000 ppm (corresponding to about 0, 83.9, 171.5, 373.0, 556.1 mg/kg bw/day in males, 0, 92.3, 192.9, 409.8, 617.4 mg/kg bw/day in females). Eight male and 8 female rats per group received PHMB for a period of at least 90 days (terminal kill) and the remaining 4 males and 4 females per group were killed after 29 days (interim kill). The test diets were fed continuously throughout the study. The animals were examined for mortality and clinical signs including behaviour. Ophthalmology was carried out in all animals from the control and highest dose groups during the week prior to termination. Body weight and food

consumption were determined at regular intervals. Urine samples were collected from each rat in the week prior to termination. At the end of each dosing period, the rats were killed and blood samples were taken for haematology and clinical pathology. All animals surviving to termination were anesthetised, killed by exsanguination and subjected to a complete necropsy. Kidneys and liver were weighed. Liver, kidney, stomach, duodenum, jejunum, ileum, cecum, colon, rectum and any macroscopically abnormal tissue from control and high dose group interim kill animals, only, were processed for microscopic examination. Statistical analyses were performed by means of analysis of variance and two-sided Student's T-test.

Results:

The dietary administration of 2000, 4000 and/or 6000 ppm was associated with a clear treatment-related effect on body weight for males and/or females. An associated reduction in food consumption was also apparent for males and females during the first week of treatment. The effects on growth were reflected in altered nutritional status, as indicated by changes in several clinical chemistry parameters and reduced urinary protein. There were also findings in several hematological parameters, indicative of slight hemoconcentration, at these dose levels. The kidney was identified as a target organ. Renal functional change in the form of decreased urine volume and increased specific gravity, was noted at doses of 2000, 4000 or 6000 ppm for interim and terminal kill animals being more marked in males. Histopathological changes in the kidney, including interstitial mononuclear cell infiltration and hypertrophy of terminal collecting ducts and pelvic epithelium, were recorded at day 29 for animals which received 6000 ppm. However, treatment-related effects on kidney weight were not apparent. At terminal kill, a clear treatment-related increase in kidney weight was apparent for males at 4000 or 6000 ppm but as histopathological examination of the kidneys from these animals was not performed, the toxicological significance was considered as not clear. Treatment-related increases in plasma alkaline phosphatase, alanine transaminase and/or aspartate transaminase activities, seen for males and for females which received 1000, 2000, 4000 or 6000 ppm at interim and/or terminal kill, were suggestive of an effect on the liver. However, no treatment-related effects on liver weight or histopathological changes in the liver were seen at interim kill. Reduced liver weight, seen for females at 6000 ppm at terminal kill, was considered likely to reflect the reduced growth of these animals and not to be due to a direct effect of PHMB on the liver. As only limited histopathological examination was performed, the toxicological significance of the macroscopic changes in the gastrointestinal tract, liver and hepatic lymph node in some terminal kill animals at 6000 ppm could not be assessed.

Conclusion:

The dietary administration of PHMB to rats at dose levels of 0, 1000, 2000, 4000 and 6000 ppm over a period of up to 90 days led to a reduction in body weight at ≥2000 ppm. The kidney was identified as the target organ for animals receiving 6000 ppm. The authors concluded that the extent of the body weight changes at 4000 or 6000 ppm would preclude the use of these dietary levels in studies of longer duration. It was concluded that a dose level of 2000 ppm would be suitable for use as the high dose level in the subsequent dietary long-term study.

With respect to the observed effects, the NOAEL was 1000 ppm (corresponding to about in 84/92 mg/kg bw/day in male and female rats) under the condition of this study.

<u>91-day dietary range-finding study in mice:</u>

Study Design:
Reference: Ref 59, 1993c

Date of report:	1993
Guideline/method:	Range-finding study with an extent comparable to OECD 408 but fewer parameters in haematology, clinical pathology and pathology
Species/strain:	Mouse/ C57Bl/10J_fCD-1
Group size:	15 male and 15 female animals per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	D4097 (Vantocil P, 20.2% aqueous PHMB solution)
Dose levels:	0, 1000, 2000, 4000 ppm (corresponding to about 0, 162, 328, 736 mg/kg bw/day in males, 0, 224, 445, 963 mg/kg bw/day in females) and 6000 ppm (terminated due to high mortality)
Route:	Oral (diet)
Exposure period:	91-days (terminal kill, 10 animals per sex and group)) 29-days (interim kill, 5 animals per sex and group)
Exposure frequency:	daily
GLP:	Yes
Published:	No

Material and methods:

The subchronic toxicity of PHMB (Vantocil P, batch: D4097, 20.2% aqueous PHMB solution) was examined within a range-finding study for a subsequent long-term feeding study in C57Bl/10J_fCD-1 mice. Groups of 15 male and 15 female mice were fed diets containing PHMB at dose levels of 1000, 2000, 4000 ppm (corresponding to about 0, 162, 328, 736 mg/kg bw/day in males, 0, 224, 445, 963 mg/kg bw/day in females) and 6000 ppm. However, due to high mortality this dose was prematurely terminated. Ten male and 10 female animals per group received PHMB for a period of at least 91 days (terminal kill) and the remaining 5 males and 5 females per group were killed after 29 days (interim kill). The test diets were fed continuously throughout the study. The animals were examined for mortality and clinical signs including behaviour. Body weight and food consumption were determined at regular intervals. All animals surviving to termination were anesthetised, killed by exsanguination and subjected to a complete necropsy. Kidneys and liver were weighed. Liver, kidney, stomach, duodenum, jejunum, ileum, cecum, colon, rectum and any macroscopically abnormal tissue from control and 4000 ppm group interim kill animals, only, were processed for microscopic examination. Statistical analyses were performed by means of analysis of variance and two-sided Student's T-test.

Results:

The dietary administration of 6000 ppm caused marked toxicity characterised by continued body weight loss and clinical signs of subdued behaviour, hunched posture and shaking and therefore this group was killed for humane reasons by day 11. A marked effect on body weight gain was noted for males and females at 4000 ppm, together with an initial reduction in food utilisation and food consumption for males only. For males at 2000 ppm, reduced body weights were noted initially during the first 5 days of the study. There were no treatment-related effects on liver or kidney weights and no gross or histo-pathological finding.

Conclusion:

The dietary administration of PHMB to mice at dose levels of 0, 1000, 2000, 4000 and 6000 ppm over a period of up to 91 days caused severe toxicity at 6000 ppm. The lower dose levels of 2000 and 4000 ppm led to a clear reduction in body weight. At target organ for systemic toxicity was not identified. The authors concluded that the dose level of 4000 ppm can be considered as the maximum tolerated dose level to be used in the subsequent long-term feeding study. With

respect to the observed effects the NOAEL was 1000 ppm (corresponding to about in 162/224 mg/kg bw/day in males/females) under the condition of this study.

In an older oral subchronic study in rats with low reliability each 25 male and 25 female Wistar rats received PHMB (Antibacterial 9073, batch: WEM/G/680, 25% aqueous PHMB solution) at dose levels of 0, 2500 and 5000 ppm via their diet over a period of 90 days. There was no mortality at any dose level. The dietary administration of 5000 ppm led to a retardation of body weight gain most likely due to reduced food consumption owing to decreased palatability. At this dose level, deposits of an iron-pigment in the liver was noted in individual female rats. The dose level of 2500 ppm was tolerated without any finding (Ref 47, 1966a).

There exists also a 13-week drinking water palatability study with PHMB (Vantocil P, batch: D4097, 20.2% aqueous PHMB solution) in mice. This study can be considered of low reliability as no haematology, clinical chemistry nor histopathology were performed. The palatability was tested with increasing the dose levels in a stepwise progression. Each 10 C57 Bl mice per sex per group received PHMB in the drinking water at initial dose levels of 0 and 0.1 mg/mL respectively. After one week, the dose level for the treated group was increased to 0.3 mg/mL, and after 2 weeks on study, the dose level was increased to 0.6 mg/mL and maintained for the remainder of the study. The increase in the dose level of PHMB in a stepwise progression from 0.1 - 0.6 mg/mL led to an initial loss of body weight and reduction in water consumption at each dose level. Although these initial changes are considered largely to reflect a palatability effect of PHMB, there was no consistent effect between males and females and the three dose levels. Overall, the dose-related reduction in water consumption indicated clearly a decrease in palatability of the selected PHMB dose levels, when applied via the drinking water. In addition to the results of the subacute 28-day study in mice mentioned above in chapter 6.5.1.1, these results are an additional confirmation that application of PHMB via the drinking water can be considered as not appropriate for long-term application (Ref 57, 1992c).

In an older oral subchronic study in Beagle dogs with low reliability each 4 male and 4 female dogs per group received PHMB (Antibacterial 9073, batch: WEM/G/680, 25% aqueous PHMB solution) at dose levels of 0, 5500 and 11000 ppm in their daily pelleted diet over a period of 90 days. There was no mortality or any sign of clear systemic toxicity at any dose level. The only finding observed was a slight reduction in neutrophils of questionable relevance (Ref 48, 1966b).

6.5.3 Chronic toxicity

6.5.3.1 Chronic oral toxicity

Two-year combined chronic toxicity/carcinogenicity studies in rats

The chronic toxicity of PHMB (Vantocil P, batch: D4097, 20.2% aqueous PHMB solution) was examined within a combined chronic toxicity/carcinogenicity in Alpk:APfSD (Wistar derived) rats (Ref 61, 1996, Ref 84, 1993, Ref 13, 1996, Ref 11, 2002a). Therefore, a detailed presentation on materials and methods, results and conclusion also in respect to chronic toxicity is provided in chapter 6.7.1.

One year toxicity study in dogs

Study Design:

Reference:	Ref 60, 1995; Ref 84, 1993
Date of report:	1995
Guideline/method:	Comparable to OECD 452
Species/strain:	Dog/Beagle

Group size:	4 male and 4 female animals per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	D4097 (Vantocil P, 20.2% aqueous PHMB solution)
Dose levels:	0, 300, 1500, 4500 ppm up to weeks 11/12 and reduced to 3000 ppm thereafter (corresponding to about 0, 11, 54, 169/108 mg/kg bw/day)
Route:	Oral (diet)
Exposure period:	One year
Exposure frequency:	daily
GLP:	Yes
Published:	No

Material and methods:

PHMB (Vantocil P, batch: D4097, 20.2% aqueous PHMB solution) was tested for its chronic toxicity in Beagle dogs. Each 4 males and 4 females per group received PHMB at dose level of 0, 300, 1500 and initially 4500 ppm admixed in their diet for a period of one year. The high dose of 4500 ppm was reduced to 3000 ppm from weeks 11/12 onward due to unexpectedly severe reactions predominantly in males as 3 out of 4 males had to be killed for humane reasons. The test diets were fed continuously throughout the study. In the absence of a reliable method for quantitative analysis of PHMB in diet, the measurement of achieved concentration, homogeneity and stability in diet was not performed. However, the homogeneity of the mixing procedure was determined using an aqueous solution of a dyestuff and was performed at approximately 6 monthly intervals. Information demonstrating that the method used produced homogenous diets was reported separately (Ref 84, 1993).

The animals were examined for mortality and clinical signs including behaviour. Body weight and food consumption were determined at regular intervals. Blood samples for hematolology and clinical pathology were taken in weeks -1, 4, 13, 26 and 52 and from intercurrent high dose males). Urine samples were taken prior to the start of the study and in weeks 26 and 52. All dogs surviving to termination and those killed intercurrently were anesthetised, killed by exsanguination and subjected to a complete necropsy. Adrenal glands, brain, epididymides, kidneys, liver, testes and thyroid glands were weighed. A comprehensive number of organs and tissues were processed, fixed, stained and examined by histopathology. Statistical analyses were performed by means of analysis of variance and Student’s T-test.

Results:

The mixing procedure was verified by analysing the homogenous distribution of an ionically similar dye to PHMB, methyl violet. The homogeneity was demonstrated for the dietary 300 and 4500 ppm preparation.

Three males receiving 4500 ppm were killed for humane reasons after showing severe and unexpected clinical signs of toxicity (including marked reddening/peeling of scrotal skin), inappetence, body weight loss and/or indications of liver impairment in the form of elevated plasma alanine transaminase and/or aspartate transaminase activities. Similar scrotal skin lesions were also apparent for the remaining high dose group dog, when receiving 4500 ppm Inappetence/body weight loss was noted in 2 females at this dose level. Following reduction of the high dose level to 3000 ppm, one female was killed for humane reasons after showing clinical signs of toxicity, including stiff/splayed gait, and elevated plasma alanine transaminase and aspartate transaminase activities. For animals surviving to termination, treatment related clinical observations (red/brown staining of coat, predominantly paws and hocks) were recorded for one female receiving 300 ppm and for all animals at 1500 or 3000 ppm, but, in the absence of any associated histopathological changes, these findings were considered to be of no

toxicological relevance. Plasma cholesterol levels were reduced for the surviving animals receiving 3000 ppm. Plasma alanine transaminase and/or aspartate transaminase activities were elevated for the surviving male, for 2/3 females receiving 3000 ppm and for one female each at 300 or 1500ppm. Low testis weight was also apparent for the surviving male at 3000 ppm. Treatment-related histopathological findings were present in the skin in the form of dermatitis of the scrotum, chin and limbs as well as in the liver, kidney (males only) and testis of animals that received 4500/3000 ppm. There were no histopathological changes in animals receiving 300 or 1500 ppm that were considered attributable to treatment. The elevated plasma alanine transaminase and aspartate transaminase activities in isolated animals receiving 300 or 1500 ppm, in the absence of any associated histopathological changes, were considered to be of no toxicological importance

Conclusion:

The dietary administration of PHMB to male and female Beagle dogs at concentration of 0, 300, 1500 and 4500/3000 ppm (corresponding to about 0, 11, 54 and 169/108 mg/kg bw/day) for one year led to severe signs of toxicity at 4500/3000 ppm in the form unequivocal clinical signs of toxicity and indications of liver impairment. Histopathological findings as a consequence of the treatment with 4500/3000 ppm occurred in the skin in the form of dermatitis of the scrotum, chin and limbs as well as in the liver, kidney (males only) and testis. The animals receiving 300 or 1500 ppm, showed only very minor effects, which were considered to be of no toxicological relevance due to the absence of corresponding histopathological findings.

The NOAEL for systemic toxicity was 1500 ppm (corresponding to about 54 mg/kg bw/day) in male and female Beagle dogs under the conditions of this study.

6.5.4 Overall conclusion on repeated dose toxicity

In subacute oral studies, designed as range-finding and/or palatability studies, predominantly body weight, food and/or water consumption were affected after administration of PHMB via the drinking water for 28 days at 0, 0.1, 0.5, .10, 2.0 mg/mL in rats or 0, 0.1, 0.3, 0.6 and 1.2 mg/mL in mice. Indications of functional impairment of the liver and kidney but no serious damage of organs were noted. A NOEL was not achieved at 0.1 mg/mL (corresponding to about 16/18 mg/kg bw/day in rats, about 21 mg/kg bw/day in mice) in these studies. However, as no histopathology was performed, the relevance of the observed findings remained uncertain and therefore these were not considered as adverse effects. The reduced palatability, which could have led to secondary effects in these drinking water studies in rats and mice, indicated that this route of exposure cannot be considered as an appropriate oral route of exposure.

The subacute administration of PHMB via the diet for 6 weeks to one dog per sex at dose levels of 0, 2500, 4000 and 5500 ppm showed signs of systemic toxicity at both high dose levels. At 5500 ppm reduced food intake and weight loss in the female was observed. At 4000 and 5500 ppm there was evidence of liver toxicity characterised by reductions in plasma cholesterol, increases in the activities of specific plasma enzymes associated with liver changes and microscopic hepatocellular changes. There was also evidence for minor kidney changes at these dose levels. The NOEL for male and female dogs was 2500 ppm (corresponding to about 90 mg/kg bw/day).

The subacute dermal administration of PHMB under an occlusive dressing at dose levels of 0, 20, 60 and 200 mg/kg bw/day for 6 h/day during a 30 day period led to no sign of systemic toxicity in male and female rats. Signs of slight to moderate skin irritation occurred in a dose-dependent manner and increase with regards to severity and duration. The NOAEL for systemic

toxicity was 200 mg/kg bw/day, the highest dose level investigated. The NOAEL for dermal irritation was 20 mg/kg bw/day.

The subacute inhalation of PHMB at concentrations of 0, 0.025, 0.25 or 2.5 mg/m³ for 6 hours per day, 5 days per week, over a period of 28 days led to substance-induced findings at 0.25 and 2.5 mg/m³ in the form of some transient but reversible histopathological changes in the larynx and trachea that were characteristic of exposure to a respiratory tract irritant. Body weight changes were present at these exposure concentrations. Some non-resolving histopathology changes in the lungs were limited to the highest exposure concentration of 2.5 mg/m³. The NOAEL for systemic toxicity was 0.025 mg/m³ in male and female rats under the condition of the study.

The dietary administration of PHMB to rats at dose levels of 0, 1000, 2000, 4000 and 6000 ppm over a period of up to 90 days led to a reduction in body weight at ≥2000 ppm. The kidney was identified as the target organ for animals receiving 6000 ppm. The NOAEL was 1000 ppm (corresponding to about in 84/92 mg/kg bw/day in males/females).

The dietary administration of PHMB to mice at dose levels of 0, 1000, 2000, 4000 and 6000 ppm over a period of up to 91 days caused severe toxicity at 6000 ppm. The lower dose levels of 2000 and 4000 ppm led to a clear reduction in body weight. At target organ for systemic toxicity was not identified. The NOAEL was 1000 ppm (corresponding to about in 162/224 mg/kg bw/day in males/females).

The dietary administration of PHMB to male and female Beagle dogs at dose levels of 0, 300, 1500 and 4500/3000 ppm for one year led to severe signs of toxicity at 4500/3000 ppm in the form of clinical signs of toxicity and indications of liver impairment. Treatment-related histopathological findings were present in the form of dermatitis of the scrotum, chin and limbs as well as in the liver, kidney and testis. The NOAEL for systemic toxicity was 1500 ppm (corresponding to about 54 mg/kg bw/day) in male and female Beagle dogs.

The dietary administration of PHMB at dose levels of 0, 200, 600 and 2000 ppm in a combined chronic toxicity/carcinogenicity study in male and female rats for a period of up to two years led to treatment-related findings only at the high dose of 2000 ppm in the form of slightly reduced survival in females, reduced body weight in both sexes and changes in clinical-chemistry indicative for a slight liver impairment. The NOAEL for chronic toxicity was 600 ppm (equivalent to about 36 and 45 mg/kg bw/day for males and females, respectively). The NOAEL of 36 mg/kg bw/day obtained in this two year feeding study in male animals will be used as the most representative lowest value for repeated dose toxicity for the calculation of the Margin of Safety (MoS).

6.6 Mutagenicity/Genotoxicity

6.6.1 Mutagenicity/Genotoxicity *in vitro*

6.6.1.1 Bacterial reverse mutation tests (Ames)

Study Design:

Reference:	Ref 14, 1989
Date of reports:	1989
Guideline:	OECD Guideline 471
Test system:	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538
Replicates:	Triplicate plates

Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	Bx2125 (Vantocil IB, 19.6% aqueous PHMB solution)
Concentrations:	Range-finding (TA100 only): ±S9 mix: 0, 1.6, 8.0, 40, 200, 1000, 5000 µg/plate Experiment I+II: ±S9 mix: 0, 0.32, 1.6, 8.0, 40, 200, 500 µg/plate
Vehicle:	DMSO
Positive Controls:	-S9 mix: TA 100, TA1535: N-methyl-N-nitro-N-nitrosoguanidine (MNNG), 1 – 5 µg/plate TA98: Daunorubicin (DR), 0.2 – 1.0 µg/plate TA1537: Acridine mutagen ICR191, 0.5 – 2.0 µg/plate TA1538: 4-nitro-O-phenylenediamine (4-NPD), 1.0 – 5.0 µg/plate +S9 mix: all strains: 2-aminoanthracene (2-AA), 0.2 – 1.0 µg/plate
Negative controls:	untreated and vehicle control
GLP:	Yes
Published:	No

Material and methods:

PHMB (batch: Bx2125, 19.6% aqueous PHMB solution) was tested for mutagenicity in the reverse mutation assay on bacteria with and without metabolic activation (S9 mix prepared from Aroclor 1254 induced liver of Alderley Park rats). PHMB was dissolved in DMSO, which was also used as vehicle control. In a range-finding test, PHMB was investigated over a dose range of 1.6 – 5000 µg/plate in *Salmonella typhimurium* strain TA100 with and without metabolic activation only. Due to the observed concentration-dependent bacteriotoxicity at ≥200 µg/plate, the *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 in the main study were exposed to the test substance (dissolved in DMSO) at concentrations ranging from 0.32 - 500 µg/plate. For control purposes, the negative, vehicle (DMSO) and positive controls (MNNG, DR, Acridine mutagen ICR191, 4-NPD, 2-AA) were also investigated. Two independent experiments were performed.

Results:

Bacteriotoxicity was observed at ≥200 µg/plate. The number of revertant colonies did not differ between plates containing the test substance and those containing the negative controls either with or without metabolic activation. Although slight effects were observed in strain TA98 (+S9) these were not consistently observed in all experiments with this strain. The positive controls induced large numbers of revertant colonies in each bacterial strain demonstrating the sensitivity and suitability of the test system.

Conclusion:

PHMB did not induce gene mutations by base pair changes or frame shifts in the genome of the bacterial strains used in this Ames test either in the presence or absence of S9-mix. Thus, PHMB was shown to be non-mutagenic in this bacterial gene mutation test. There exists also a previous Salmonella reverse mutation assay performed with PHMB (Vantocil IB, batch: AGDM 2253/77, 20% aqueous PHMB solution) in *Salmonella typhimurium* strains (TA98, TA100, TA1535, TA1537, TA1538) with and without metabolic activation. PHMB was toxic at a dose of 333.3 mg per plate, particularly in strains TA98, TA100, and TA1535 and was shown to be weakly

mutagenic in strain TA1538 in the absence of metabolic activation. However, as this study was performed prior to the implementation of GLP and the report contained insufficient information to determine the adequacy of the test, the report has to be considered as not reliable (Ref 51, 1979). In a subsequently performed *Salmonella typhimurium* reverse mutation assay with the same batch of PHMB and performed under comparable conditions (*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538, ± metabolic activation), PHMB was shown to be non-mutagenic (Ref 111, 1980).

6.6.1.2 *In vitro* mammalian cell gene mutation tests

Study Design:

Reference:	Ref 50, 2002
Date of report:	2002
Guideline/Method:	OECD 476
Test system:	Mouse lymphoma cells (L5178Y)
Replicates:	Duplicate cultures, two tests
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	1202 (P20D, 20% aqueous PHMB solution)
Concentrations:	±S9 mix: 0, 12.5, 25, 50, 100 µg/mL
Exposure:	Test 1: 3 hours (±S9 mix) Test 2: 20 hours (-S9 mix), 3 hours (+S9 mix)
Vehicle:	DMSO
Positive Control:	-S9 mix: methyl methanesulfonate (MMS), 2 and 10 µg/mL +S9 mix: Cyclophosphamide (CPA), 2 µg/mL
GLP:	Yes
Published:	No

Material and methods:

PHMB (P20D, batch: 1202, 20% aqueous PHMB solution) was tested for its potential to induce gene mutations *in vitro* in L5178Y mouse lymphoma cells at the thymidine kinase (TK) locus using the microtiter cloning technique (Trifluorothymidine resistance). Prior to main assay a pre-test was carried out in order to determine the cytoxicity of the test substance by measuring the colony-forming ability and the growth rate. The relative suspension growth (RSG) and the relative total growth (RTG) were determined. In the main assay the test item was diluted with DMSO and examined in four concentrations (12.5, 25, 50, 100 µg/mL) considering the requested cytotoxicity criteria. The main assay was performed in two independent tests. Each test was performed in 2 replicates as well as with and without metabolic activation in the form of S9 mix of Aroclor 1254 induced Sprague-Dawley rat liver. Exposure to the cells in test 1 was 3 hours, while in the test 2 the cells were exposed for 20 hours without metabolic activation and for 3 hours with S9 mix. Thereafter, the cells were washed and resuspended in order to determine their survival rate and to allow the phenotypic expression of the mutation. At the end of the expression time, the cells are exposed to trifluorothymidine (TFT). The treated cultures were maintained 48 - 72 hours at 37° C to allow phenotypic expression of induced mutations. The mutant frequency was determined by seeding the cells in medium containing the selective agent (TFT) to detect mutant cells, and in complete culture medium (Dulbecco's modified Eagle's medium (DMEM)) without TFT to determine cloning efficiency. All colonies considering large and small colonies were scored after an incubation period of 10 - 14 days at 37° C in a humidified atmosphere containing 5 % CO_2. The number of mutant colonies in selective medium (DMEM plus TFT) was adjusted by the number of colonies in non-selective medium to derive the mutant frequency.

Negative control (DMSO) and positive controls were (MMS, -S9 mix, 2 and 10 µg/mL; CPA, +S9 mix, 2 µg/mL) were tested in parallel.

Results:

The observed cytotoxicity was in the requested range as the relative total growth (RGT) values at any concentrations tested (±S9 mix) were compatible with the assay requirements (down to 23%). At concentrations of 50 and 100 µg/mL, PHMB was shown to be more cytotoxic than positive controls. A sufficient number of surviving colonies for evaluation was observed at the tested concentrations in the range between 12.5 – 100 µg/mL with and without S9 mix.
No increase in the mutation frequency was observed at any PHMB concentration irrespectively of the presence or absence of metabolic activation. The positive controls MMS and CPA induced a distinct increase in mutant colonies with (CPA) and without (MMS) metabolic activation indicating that the test was sensitive and valid.

Conclusion:

Under the conditions of the study, PHMB did not induce gene mutations in L5178Y mouse lymphoma cells at the thymidine kinase (TK) locus using the microtiter cloning technique in the absence and presence of metabolic activation up to cytotoxic concentrations and was therefore considered to be not mutagenic.

Study Design:

Reference:	Ref 1, 1981
Date of report:	1981
Guideline/Method:	Comparable to OECD 476
Test system:	Mouse lymphoma cells (P388)
Replicates:	5 replicate cultures
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	ADGM/1021/79 (Vantocil P, 20% aqueous PHMB solution)
Concentrations:	-S9 mix: 25, 250, 500, 1000, 2000 µg/mL +S9 mix: 25, 250, 1000, 2000 µg/mL
Vehicle:	DMSO
Positive Control:	±S9 mix: methyl methanesulfonate (MMS), 32.5 and 65 µg/mL
GLP:	Yes
Published:	No

Material and methods:

PHMB (Vantocil P, batch: ADGM/1021/79, 20% aqueous PHMB solution) was tested for its potential to induce gene mutations *in vitro* in P388 mouse lymphoma cells (thymidine kinase (TK)). The assay was performed in five replicate cultures. PHMB was diluted with DMSO and was added to the cell cultures for 30 minutes at dose levels of between 25 – 2000 µg/mL with and without metabolic activation. The metabolic activation system was obtained from Aroclor 1254 induced liver of male Sprague-Dawley rats. During the treatment period, the cell suspensions in the test tubes were rotated on a flat bed test tube roller. After exposure, the cells were washed and maintained in medium for an expression time between 68 – 72 hours at 37° C. Thereafter, 5-iodo-2'-deoxyuridine (IUdR) was added as selective agent and molten agar was added to the test tubes. The mixtures were shaken, dispensed into a petri dish. The petri dishes were placed in a CO_2 incubator until colony formation. The colonies were counted using a dissecting microscope and the mean of 5 replicates was determined.

Negative controls (DMSO) and positive controls (MMS, ±S9 mix, 32.5 and 65 µg/mL) were tested in parallel.

Results:

The concentration of 2000 µg/mL was cytolethal and clear cytotoxicity was noted at 1000 µg/mL in the presence and absence of metabolic activation. A sufficient number of surviving colonies for evaluation was observed at concentrations in the range between 25 – 1000 µg/mL with and without S9 mix. No increase in the mutation frequency was observed at any PHMB concentration irrespectively of the presence or absence of metabolic activation. The positive control MMS induced a distinct increase in mutant colonies with and without metabolic activation indicating that the test was sensitive and valid.

Conclusion:

Under the conditions of the study, PHMB did not induce gene mutations at the TK locus in P388 mouse lymphoma cells in the absence and presence of metabolic activation up to cytotoxic concentrations and was therefore considered to be not mutagenic.

6.6.1.3 *In vitro* mammalian cell chromosomal aberration tests

Study Design:

Reference:	Ref 62, 1989
Date of report:	1989
Guideline/method:	OECD 473
Test system:	Cultured human peripheral blood lymphocytes from two volunteers (male and female)
Replicates:	Duplicate cultures, two independent experiments
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	Bx2125 (Vantocil IB, 19.6% aqueous PHMB solution)
Concentrations:	-S9 mix: 0, 5.0, 25, 50 µg/mL (both volunteers) +S9 mix: 0, 25, 100, 187.5 µg/mL (volunteer 1) +S9 mix: 0, 25, 100, 250 µg/mL (volunteer 2)
Solvent/negative control:	Physiological saline solution
Positive Controls:	-S9 mix: Mitomycin C (MMC), 0.5 µg/mL +S9 mix: Cyclophosphamide (CPA), 100 µg/mL
GLP:	Yes
Published:	No

Material and methods:

The potential of PHMB (batch: Bx2125, 19.6% aqueous PHMB solution) to induce chromosomal aberrations in mammalian cells was examined in cultured human peripheral blood lymphocytes from two volunteers. The assay was performed with and without metabolic activation (S9 mix). The test substance was dissolved in physiological saline solution, which was also used as solvent control. PHMB was administered to the cultures approximately 44 hours after initiation and remained in contact with the cells for 2 hours 20 minutes to 3 hours 40 minutes, after which the growth medium was replaced with fresh medium. Dividing lymphocytes were arrested in metaphase by treatment with 10 µg/mL colchicine at 70 hours after culture initiation. At 72 hours the cells were then subjected to hypotonic treatment for 12 minutes in 0.075M KC1 followed by fixation in glacial acetic acid/methanol. Slides were stained in Giemsa. Where possible, one hundred cells in metaphase were analysed from each blood culture for the incidence of structural chromosomal damage. Based on the results of a range-finding test, the highest dose levels selected for chromosomal analysis were 50 µg/mL for both volunteers in the

absence of S9-mix, and in the presence of S9-mix, 187.5 and 250 µ/mL for volunteers 1 and 2, respectively. In each instance the maximum dose level was determined by a reduction in mitosis of approximately 50-80%.

The appropriate dose levels selected for chromosomal analysis were 50, 25 and 5 µg/mL for both volunteers in the absence of S9-mix; 250, 100 and 25 µ/mL for volunteer 2 in the presence of S9-mix; 187.5, 100 and 25 µ/mL for volunteer 1 in the presence of S9-mix. Cyclophosphamide (0.5 µg/mL) for the non-activation set and Cyclophosphamide (100 µg/mL) requiring activation served as positive control substances. A negative (solvent) control (physiological saline solution) was also included in the test.

Results:

PHMB led to a dose-related suppression of cell growth and/or cell death. This activity was partly reflected in the mitotic index determinations. Volunteer 2 was affected more than volunteer 1. In addition to the depression in mitotic index there was also a reduction in the overall numbers of cells present. For some dose levels this resulted in fewer than the target of 200 metaphases. However, sufficient cells were analysed for volunteer 2 at each dose level to allow a meaningful assessment, and considering the data from volunteers 1 and 2 together. The overall mitotic activity was reduced by approximately 50-80%. In any case, PHMB could be examined for clastogenicity at dose levels up to and including those causing clear cytotoxic effects. No statistically or biologically significant increases in chromosomal damage were seen in any cultures treated with PHMB in either donor, either in the presence or absence of metabolic activation. The sensitivity and validity of the test system used was demonstrated by the expected induction of a significantly increase in the proportion of cells with structural aberrations with the positive controls.

Conclusion:

PHMB did not induce chromosome aberrations in cultured human peripheral blood lymphocytes, when tested in the absence and presence of metabolic activation up to cytotoxic concentrations.

This result is in line with a previously performed *in vitro* cytogenetic study with PHMB (Vantocil P, batch: ADGM/1021/79, 20% aqueous PHMB solution). This study was also performed with human lympocytes under comparable conditions as the above study but with peripheral blood lymphocytes of only one male donor and without metabolic activation. Thus, it can be considered as supportive information. PHMB was diluted in water and tested at concentrations of 1, 5, 10, 20 and 50 µg/ml. The vehicle and Mitomycin C (0.5 µg/mL) was tested in parallel as negative and positve controls, respectively. PHMB did not induce any dose-related or statistically significant increase in chromosome damage up to concentrations, which led to decreases in the mitotic index. Thus, PHMB was shown to exhibit no clastogenic potential in human lymphocytes exposed *in vitro* without a metobolic activation system (Ref 98, 1981).

6.6.2 Mutagenicity/Genotoxicity *in vivo*

6.6.2.1 Micronucleus assay

Study Design:

Reference:	Ref 97, 1989
Date of report:	1989
Guideline/Method:	OECD 474
Species/strain:	Mouse/C57Bl

Group size:	5 males, 5 females per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	Bx2125 (Vantocil IB, 19.6% aqueous PHMB solution)
Dose levels:	0, 250, 400 mg/kg bw
Exposure:	Single application
Route:	Oral (gavage)
Vehicle:	Double deionised water
Sacrifice Times:	24, 48, 72 h after application
Positive control:	Cyclophosphamide (CPA, 65 mg/kg bw)
GLP:	Yes
Published:	No

Material and methods:

PHMB (batch: Bx2125, 19.6% aqueous PHMB solution) was examined for its cytogenetic potential in vivo in the micronucleus test in male and female C57Bl mice after a single oral application by gavage. Each 5 animals per sex received PHMB diluted in double deionised water at dose levels of 0, 250 and 400 mg/kg bw. The animals were killed at 24, 48 and 72 hours after dosing and bone marrow was obtained, processed, and bone marrow suspension cells were spread onto glass slides. The bone marrow cell preparations were stained with polychrome methylene blue and eosin to visualise the various cell types. For each animal, initially 1000 polychromatic erythrocytes were scored for the presence of micronuclei. The portion of polychromatic erythrocytes to total erythrocytes was recorded per 1000 erythrocytes. An extended evaluation of an additional 2000 polychromatic erythrocytes (PCE) per animal was performed at the 400 mg/kg bw/day group and control animals at all three sampling time points. Cyclophosphamide (65 mg/kg bw/day) was used as positive control substance at 24 hours sampling time only. The incidence of micronucleated polychromatic erythrocytes (MPE) and percentage polychromatic erythrocytes (PCE) were considered by analysis of variance (ANOVA), regarding each combination of sampling time, dose level and sex as a separate group.

Results:

No mortality occurred in any male or female mice at any dose level. The high dose of 400 mg/kg bw/day led to a reduction in polychromatic erythrocytes as an indication that the maximum tolerated dose level was investigated. No statistically significant increase in the frequency of MPE was observed at either dose level of PHMB at any of the sampling times investigated, when the sexes were combined. However, when the sexes were considered separately, a small statistically significant increase in MPE was apparent in male mice at the 400 mg/kg bw/day dose level at the 48 hour sampling time. It was noted that the value obtained for the control male group at 48 hours was low compared with the control values at the other two sampling times and that there was also a significant degree of animal variability in these groups. Therefore, a further 2000 PCE were examined for the male 400 mg/kg bw/day group and the male negative control group at all sampling times. Following the extended counts, there was no difference between the test and control male animals at the 48 hour sampling time and no statistically significant differences were obtained whether the extended counts were analysed alone (as 2000 PCE) or combined with the original counts (as 3000 PCE). The sensitivity of the test system was shown since Cyclophosphamide as the positive control substance caused chromosomal aberrations.

Conclusion

Under the conditions of the study, PHMB did not induce a significant or biologically relevant increase in micronucleated polychromatic erythrocytes in male or female mice, when tested up to

the maximum tolerated dose level. Thus, PHMB was shown to exhibit no clastogenic potential *in vivo*.

6.6.2.2 UDS assay

Study Design:

Reference:	Ref 112, 1989
Date of report:	1989
Guidelines:	Unscheduled DNA synthesis (UDS) assay according to Ashby et al., 1983, 1985 comparable to OECD 486
Species/strain:	Rat/Alpk:APfSD (Wistar derived)
Replicates:	Two independent experiments
Group size:	2 - 3 male animals per dose and experiment
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	Bx2125 (Vantocil IB, 19.6% aqueous PHMB solution)
Dose levels:	0, 750, 1500 mg/kg bw
Exposure:	Single application
Route:	Oral (gavage)
Vehicle:	PHMB: Double deionised water Positive control: corn oil
Positive control:	6-p-dimethylaminophenyylazobenzthiazole (6-BT, 40 mg/kg bw)
Exposure period:	4, 12 h after application
GLP:	Yes
Published:	No

Material and methods:

PHMB (batch: Bx2125, 19.6% aqueous PHMB solution) was assessed for its potential to induce DNA-damage and -repair in the in vivo/in vitro UDS test using rat hepatocytes. The choice of the dose levels was based on the acute oral toxicity study reported in section 5.1.1; Ref 63, 1979a). The test substance diluted with double deionized water was administered orally (by gavage) at dose levels of 750 and 1500 mg/kg bw for a period of 4 or 12 hours to groups of 2 - 3 male Alpk:APfSD (Wistar derived) rats per dose and experiment. Two independent experiments were carried out. The application volume was 10 ml/kg bw. After the treatment periods, the animals were sacrificed and liver perfusion was carried out. From each animal primary hepatocytes cultures were established and exposed for 4 hours to [^{3}H]-thymidine, which is incorporated into the DNA, if UDS occurs. Following the [^{3}H]-thymidine exposure period, the cells were washed, fixed, dried and mounted on cover slips, coated with photographic emulsion and stored in darkness for14 days at 4°C. Thereafter, the slides were developed at room temperature and stained. The net nuclear grain counts were determined by counting 100 cells per animal. The appropriate reference mutagen 6-p-dimethylaminophenyylazobenzthiazole (6BT, 40 mg/kg bw) was used as positive control.

Results:

Overt clinical findings in the form of excessive salivation and subdued attitude were observed at 1500 mg/kg bw. Treatment with 750 or 1500 mg/kg bw of PHMB revealed no UDS induction in the hepatocytes of the treated animals as compared to the corresponding vehicle controls. The reference mutagens revealed distinct increases in the number of nuclear and net grain counts and thus, confirmed the sensibility and validity of the test system.

Conclusion:

Under the experimental conditions reported, the test substance did not induce DNA-damage, i.e. no increased repair synthesis in the hepatocytes of the treated rats. Therefore, PHMB was shown to be non-genotoxic in this *ex vivo/ in vitro* UDS test system when tested up to a dose level inducing overt clinical findings.

6.6.3 Overall conclusion on mutagenicity/genotoxicity

PHMB was tested for its mutagenic/genotoxic potential in a range of validated and scientifically reasonable studies *in vitro* and *in vivo* covering all relevant endpoints of mutagenicity/genotoxicity. On the basis of the available data, PHMB is not considered to be genotoxic. This is supported by the Risk Assessment Committee (RAC) of ECHA who concluded that PHMB has no genotoxic potential.

Overall, PHMB was negative in the Ames test with and without metabolic activation when tested up to bacteriotoxic concentrations. Despite being a broad-spectrum preservative with biocidal properties, the observed cytotoxicity of PHMB was not too excessive to prevent the evaluation of the mutagenic potential. It produced only moderate toxicity in Salmonella strains. An adequate dose range from 0.32 to 500 µg/plate was investigated and concentration-dependent cytotoxicity started only at concentrations as high as 200 µg/plate with and without S9.

PHMB did not induce gene mutations *in vitro* at the TK locus in P388 mouse lymphoma cells or more recently in L5178Y mouse lymphoma cells using the microtiter cloning technique in the absence and presence of metabolic activation up to cytotoxic concentrations. The substance did not induce chromosome aberrations *in vitro* in cultured human peripheral blood lymphocytes, when tested in the absence and presence of metabolic activation up to cytotoxic concentrations.

In vivo, there was no induction of micronucleated polychromatic erythrocytes in male or female mice, when tested up to the maximum tolerated dose level and thus, PHMB was shown to exhibit no clastogenic potential *in vivo*. The substance did not induce DNA-damage in the hepatocytes of the rats and was shown to be non-genotoxic in the *in vivo/in vitro* UDS test system, when tested up to a dose level inducing overt clinical findings indicative for systemic availability and systemic toxicity.

6.7 Carcinogenicity

6.7.1 Oral studies

Combined chronic toxicity/carcinogenicity study in rats:

Study Design:

Reference:	Ref 61, 1996, Ref 84, 1993, Ref 13, 1996, Ref 11, 2002a (HCD in rats)
Date of report:	1996
Guideline/method:	Comparable to OECD 453
Species/strain:	Rat/Alpk:APfSD (Wistar derived)
Group size:	64 male and 64 female animals per group (main group: 52 animals/sex/group, interim kill group: 12 animals/sex/group)
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	D4097 (Vantocil P, 20.2% aqueous PHMB solution)
Dose levels:	0, 200, 600, 2000 ppm (corresponding to about 0, 12.1, 36.3, 126.1 mg/kg bw/day in males, 0, 14.9, 45.3, 162.3 mg/kg bw/day in females)

Route:	Oral (diet)
Exposure period:	Two years
Exposure frequency:	daily
Interim kill:	Week 52
GLP:	Yes
Published:	No

Material and methods:

PHMB (Vantocil P, batch: D4097, 20.2% aqueous PHMB solution) was examined for chronic toxicity and carcinogenicity in a combined study in Alpk:APfSD (Wistar derived) rats. Each 64 males and 64 females per group received PHMB at dose levels of 0, 200, 600 and 2000 ppm (corresponding to 0, 12.1, 36.3, 126.1 mg/kg bw/day in males, 0, 14.9, 45.3, 162.3 mg/kg bw/day in females) in their diet for a period of up to two years. Twelve rats of each sex and group were designated for interim kills after 52 weeks and the remaining animals were killed after 105 weeks. The test diets were fed continuously throughout the study. In the absence of a reliable method for quantitative analysis of PHMB in diet, the measurement of achieved concentration, homogeneity and stability in diet was not performed. However, the homogeneity of the mixing procedure was determined using an aqueous solution of a dyestuff and was performed at approximately 6 monthly intervals. Information demonstrating that the method used produced homogenous diets was reported separately (reference: Ref 84, 1993). Eight male and 8 female microbiological sentinel animals were included in the study to provide information about the microbiological status of the experimental mice. They were fed either control diet or diet containing 2000 ppm. But these animals were not an integral part of the study.

The animals were examined for mortality and clinical signs including behaviour. Body weight and food consumption were determined at regular intervals. Ophthalmology was carried out in all animals prior to the start of the study and all surviving animals from the control and highest dose groups at week 52 and during the week prior to termination. Blood samples for hematolology and clinical pathology were collected in weeks 14, 27, 53, 79 and 105. Urine samples were collected in weeks 13, 26, 52, 78 and 104. All animals requiring euthanasia prior to study termination together with all rats killed at interim and terminal kill in weeks 53 and 105 were anesthetised, killed by exsanguination and subjected to a complete necropsy.

Testes, kidneys, adrenal glands, liver and brain were weighed. A comprehensive number of organs and tissues were processed, fixed, stained and examined by histopathology. Statistical analyses were performed by means of analysis of variance, two-sided Student’s T-test, Fisher’s exact test, Cochran-Armitage test, Kaplan-Meier survival estimates and Peto time-to tumor prevalence analysis.

Results:

The mixing procedure was verified by analysing the homogenous distribution of an ionically similar dye to PHMB, methyl violet. The homogeneity was demonstrated for the dietary 200 and 2000 ppm preparation.

There was no effect on mortality rates in males at any dose level but slightly reduced survival was noted in females receiving 2000 ppm during the second year of this study. At 2000 ppm there were treatment- related reductions in body weight being more pronounced in females. During the initial phase of the study, food consumption was reduced for both sexes at 2000 ppm, although slightly increased food consumption was recorded for females at this dose level during the second year of the study. There were no treatment-related clinical signs, ophthalmoscopic findings or effects on any haematological or urinalysis parameters throughout the study. Slightly raised plasma alkaline phosphatase activity, predominantly in females receiving 2000 ppm, and a

slightly increased incidence of hepatocyte fat and spongiosis hepatis in males at this dose level were considered as a mild effect in the liver. The pathological examination showed no non-neoplastic nor neoplastic findings at any dose level in either sex.

There was an increased incidence of haemangiosarcoma in females receiving 2000 ppm, which was not statistically significant in the Fisher's Exact Test, but gave positive results in the trend test. As this tumor type was not present in control females on this study, and is relatively rare in the Alpk:APfSD strain of rat, an external peer review and a Pathology Working Group (PWG) review was subsequently conducted to confirm the diagnosis of the vascular neoplasms and to provide assistance in the interpretation of this equivocal result. Following the PWG review, the consensus diagnosis for the incidence of rats with haemangioma and/or haemangiosarcoma in the liver was as follows:

Table 6.7.1.1 Total incidence of rats with haemangioma/haemangiosarcoma in the liver following pathology working group review (Ref 13, 1996)

	0 ppm	**200 ppm**	**600 ppm**	**2000 ppm**
Males				
601. Haemangioma	602. 0/64	603. 0/64	604. 0/64	605. 2/64
606. Haemangiosarcoma	607. 0/64	608. 0/64	609. 0/64	610. 0/64
611. Females				
612. Haemangioma	613. 0/64	614. 0/64	615. 0/64	616. 2/64
617. Haemangiosarcoma	618. 0/64	619. 0/64	620. 0/64	621. 1/64

The incidence of vascular neoplasms in the liver in this study was low and predominantly benign. In the absence of histopathological evidence of an increased incidence of non-neoplastic vascular changes in the liver of animals receiving 2000 ppm, it was considered that the slightly increased incidence of vascular neoplasms of the liver in these animals was incidental due to biological variation and thus, not related to the administration of PHMB. There were no other changes that could be attributed to administration of PHMB.

Conclusion:

The dietary administration of PHMB at dose levels of 0, 200, 600 and 2000 ppm in this combined chronic toxicity/carcinogenicity study in male and female Alpk:APfSD (Wistar derived) rats for a period of up to two years led to treatment-related findings only at the high dose of 2000 ppm in the form slightly reduced survival in females, reduced body weight in both sexes and changes clinical-chemistry indicative for slight liver impairment. Thus, the criteria for a maximum tolerated dose (MTD) were achieved. Finally, there was no evidence of a carcinogenic effect associated with the administration of PHMB for two years at dose levels of up to 2000 ppm. The no observed effect level for chronic toxicity in the rat following dietary administration of PHMB for two years was 600 ppm (equivalent to about 36 and 45 mg/kg bw/day for males and females, respectively). The conclusion of the PWG was confirmed by an independent expert, which stated that the administration of PHMB was not treatment-related to

the development of haemangiosarcomas in rats. Finally, he concluded that PHMB can be considered as not carcinogenic in either rats or mice, specifically it does not induce haemangiosarcomas in these species at doses acceptable for long-term bioassays (Ref 22, 2009).

More recently, there was a scientific advisory panel (SAP) review on PHMB covering carcinogenicity studies in rats and mice, pathology working group reviews, regulatory responses and mode of action studies. Based on the review of all available data it was concluded that all group differences in vascular tumors in rats are incidental and therefore do not indicate carcinogenic activity (Ref 83, 2009).

There exists another combined oral chronic toxicity/carcinogenicity study in rats but due to infections and less than 50% survival at the end of study dossier, this study is considered of low reliability and is presented only for the sake of completeness. Groups of 60 male and 60 female rats of an unspecified strain were fed PHMB (Baquacil SB, batches: SDC/596 and ADGM 5911, 20% aqueous PHMB solution) at dose levels of 0, 200, 1000 and 2000 ppm. The study was terminated at 124 weeks, when 80% mortality occurred. Interim kills were performed at weeks 52 and 104 weeks. There were two outbreaks of respiratory infection occurred during the study, one at 70 weeks and the other at 103 weeks. The symptoms of both infections were snuffling, rapid and laboured respiration and croaking and were more severe at 103 weeks. The incidence of numbers of animals affected was evenly distributed between groups. The incidence of mortalities was similar across all groups throughout the study. The respiratory infection at week 70 and week 103 caused a rise in the mortality rates. There were no clinical effects and no signs of systemic toxicity with the exception of a slight anaemia in the top dose at 104 weeks, no adverse findings were noted in haematology or clinical pathology. The administration of PHMB caused some growth depression in treated animals and food consumption remained lower in animals at 200 or 1000 ppm throughout the study. The long-term exposure to PHMB for almost the lifetime was not related to systemic toxicity and there was no carcinogenic effect reported up to the highest dose level of 2000 ppm (corresponding to about 85 mg/kg bw/day; Ref 4, 1977).

Carcinogenicity study in mice:

Study Design:

Reference:	Ref 87, 1996 Ref 84,, 1993, Ref 12, 2002b (HCD in mice), Ref 43, 2002
Date of report:	1996
Guideline/method:	US EPA Guideline 83-2, comparable to OECD 451
Species/strain:	Mouse/C57Bl/10J/CD-1 Alpk
Group size:	55 male and 55 female animals per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	D4097 (Vantocil P, 20.2% aqueous PHMB solution)
Dose levels:	0, 400, 1200, 4000 ppm (corresponding to about 0, 55, 167, 715 mg/kg bw/day in males, 0, 69, 217, 856 mg/kg bw/day in females)
Route:	Oral (diet)
Exposure period:	Two years
Exposure frequency:	daily
GLP:	Yes
Published:	No

Material and methods:

The possible carcinogenic potential of PHMB (Vantocil P, batch: D4097, 20.2% aqueous PHMB solution) was examined in a life-time feeding study in C57Bl/10J/CD-1 Alpk mice. Each 55 males and 55 females per group received PHMB at dose levels of 0, 400, 1200 and 4000 ppm

(corresponding to about 0, 55, 167, 715 mg/kg bw/day in males, 0, 69, 217, 856 mg/kg bw/day in females) admixed in their diet for a period of two years. The test diets were fed continuously throughout the study. In the absence of a reliable method for quantitative analysis of PHMB in diet, the measurement of achieved concentration, homogeneity and stability in diet was not performed. However, the homogeneity of the mixing procedure was determined using an aqueous solution of a dyestuff and was performed at approximately 6 monthly intervals. Information demonstrating that the method used produced homogenous diets was reported separately (Ref 84, 1993). Ten male and 10 female microbiological sentinel animals were included in the study to provide information about the microbiological status of the experimental mice. They were fed either control diet or diet containing 4000 ppm. But these animals were not an integral part of the study. The animals were examined for mortality and clinical signs including behaviour. Body weight and food consumption were determined at regular intervals. Blood samples for hematolology were collected from all animals at scheduled termination and bone marrow smears were taken from all animals and examined for females in the control and high dose groups only. All animals surviving to termination were anesthetised, killed by exsanguination and subjected to a complete necropsy. Testes, kidneys, adrenal glands, liver and brain were weighed. A comprehensive number of organs and tissues were processed, fixed, stained and examined by histopathology. Statistical analyses were performed by means of analysis of variance, two-sided Student's T-test, Fisher's exact test, Cochran-Armitage test, Kaplan-Meier survival estimates and Peto time-to tumor prevalence analysis.

Results:

The mixing procedure was verified by analysing the homogenous distribution of an ionically similar dye to PHMB, methyl violet. The homogeneity was demonstrated for the dietary 400 and 4000 ppm preparation. At 4000 ppm body weights were reduced up to 20% (males) and 15% (females) in comparison to the concurrent controls in the second year of the study. In terms of body weight gain, there was a reduction of 35-42% (males) and 22-33% (females) compared to the controls during weeks 53-79 of the study. Mortality was increased in females receiving 4000 ppm PHMB throughout the study and in males receiving 4000 ppm for the majority of the study. Therefore the 4000 ppm was clearly in excess of a maximum tolerated dose. Food consumption was increased in both sexes receiving 4000 ppm from approximately week 10 and remained higher than control throughout the study. Food utilisation was less efficient than in controls in males and females receiving 4000 ppm. The main treatment related clinical observation in males and females at the 4000 ppm was anal swelling. The first noted occurrence was in week 18 for males and week 53 for females. At termination, there was an increase in haemoglobin, hematocrit and red cell count in both sexes receiving 4000 ppm. In mice receiving 4000 ppm a variety of non-neoplastic change was seen in the liver, gall bladder and recto-anal junction.

The highest dietary concentration of 4000 ppm resulted in a markedly altered tumor profile but the significance of changes in tumor types remained uncertain in the presence of marked toxicity. At this dose level, several squamous cell carcinomas of the recto-anal junction occurred in mice of both sexes and gall bladder papillomas occurred in two males. Vascular tumors, mainly haemangiosarcomas, were increased whereas lymphosarcomas were decreased in both sexes compared to the controls. Pituitary gland adenomas were also decreased in female mice. The 1200 ppm dose level can be considered as an adequate maximum tolerated dose with reductions in body weight and non-neoplastic pathological changes at three sites; liver, recto-anal junction and gall bladder. Body weights of males and females receiving 1200 ppm were 5-6% lower than those of controls during the second year of the study. In terms of body weight gain there was a reduction of 7-14% (male) and 5-10% (female) compared to controls during weeks 53-79 of the study. In mice receiving 1200 ppm there was a variety of non-neoplastic changes in the recto-

anal junction of an inflammatory nature. In addition mice receiving 1200 ppm showed non-neoplastic changes in the liver (both sexes) and gall bladder (females only). Mice receiving 400 or 1200 ppm showed a reduced incidence of lymphosarcoma of the lympho-reticular system in comparison with controls which is considered to be treatment related. There were no other treatment related neoplastic changes. Treatment related neoplastic changes were seen in the anus and gall bladder of mice receiving 4000 ppm. In addition, mice receiving PHMB showed an altered tumor profile. The incidence of haemangiosarcomas (at any site) was increased in both sexes receiving 4000 ppm in comparison to the concurrent control group and to historical control data (reference: Ref 12, 2002b). In addition, both benign (haemangioma) and malignant (haemangiosarcoma) tumors of vascular endothelial cells are well known to occur spontaneously in mice. Published reports indicated that haemangiosarcomas occur in blood vessels of various organs at a rate of 0-14% in C57 mice. In the C57BI/10.1/CD-1 Alpk mouse, these neoplasms are a common spontaneous occurrence, frequently noted in the liver, spleen, subcutaneous tissue, lymph nodes and bone marrow (reference: Ref 12, 2002b). Conversely, there was a decrease in the number of lymphosarcomas in all males and females receiving PHMB compared to controls. Pituitary gland adenomas were also decreased in female mice. The significance of the increased tumor incidence at a dose of 4000 ppm, which was clearly in excess of a maximum tolerated dose, is unclear as doses which cause a body weight decrement from the control greater than 10-12% are known to compromise the biological interpretability of the observed response.

Conclusion:

The dietary administration of PHMB in a life-time feeding study in C57Bl/10J/CD-1 Alpk mice at dose levels of 0, 400, 1200 and 4000 ppm led to marked toxicity at the highest dose level of 4000 ppm. This dose caused marked decreases in body weight gain and was clearly well in excess of a maximum tolerated dose. The mid-dose of 1200 ppm PHMB fulfilled the criteria for a maximum tolerated dose based on reduced body weight gain and non-neoplastic pathological changes at three sites (liver, recto-anal junction and gall bladder). The highest dietary concentration used, 4000 ppm, resulted in a markedly altered tumor profile. At this dose several squamous cell carcinomas of the recto-anal junction occurred in mice of both sexes and gall bladder papillomas occurred in two males. Vascular tumors, mainly haemangiosarcomas, were increased whereas lymphosarcomas were decreased in both sexes compared to the controls. Pituitary gland adenomas were also decreased in female mice. However the changes in tumor profile were considered to be of doubtful toxicological significance as the dose level exceeded clearly the MTD. The maximum tolerated dose and no observed effect level for oncogenicity was defined at 1200 ppm.

Subsequently, a Pathology Working Group (PWG) performed a peer review of the proliferative vascular lesions on coded slides of the male and female C57Bl/10J/CD-1 Alpk mice used in the above carcinogenicity life-time study. The PWG examined slides containing sections from each of the male and female mice with a previous diagnosis of angiectasis, haemangioma, or haemangiosarcoma diagnosed by either the study pathologist and/or reviewing pathologist. The PWG examined the slides only for vascular lesions and did not consider other lesions. The diagnostic criteria used to classify the proliferative vascular lesions during this review were consistent with that currently proposed by the National Toxicology Program and the International Life Sciences Institute. The summary of the peer review is presented in the following tables:

Table 6.7.1.1 Incidence of male mice with haemangioma and/or haemangiosarcoma

Group:	0 ppm	400 ppm	1200 ppm	4000 ppm

Liver (No. Examined)	622. (	3. (2)	624. (	625. (
626. Haemangioma	627. 1	628. 1	629. 2	630. 8
631. Haemangiosarcoma	632. 3	633. -	634. 6	635. 1
636. Heart (No. Examined)	637. 0	638. (	639. (	640. (
641. Haemangioma	642. -	643. -	644. 2	645. -
646. Haemangiosarcoma	647. -	648. -	649. -	650. -
651. Spleen (No. Examined)	652. (	653. (	654. (	655. (
656. Haemangioma	657. -	658. 1	659. -	660. 3
661. Haemangiosarcoma	662. 3	663. 4	664. -	665. 2
666. Bone Marrow (No. Examined)	667. (1)	668. (1)	669. (0)	670. (1
671. Haemangioma	672. 1	673. 1	674. -	675. 1
676. Haemangiosarcoma	677. -		678. -	679. -
680. Lymph Nodes (No. Examined)	681. (0)	682. (1)	683. (0)	684. (0
685. Haemangioma		686. -	687. -	688. -
689. Haemangiosarcoma	690. -	691. -	692. -	693. -
694. Limb (No. Examined)	695. (	696. (	697. (	698. (
699. Haemangioma	700. -	701. -	702. -	703. -
704. Haemangiosarcoma	705. 1	706. -	707. -	708. -
709. Skin (No. Examined)	710. (	711. (	712. (	713. (
714. Haemangiosarcoma	715. -	716. 1	717. -	718. -
9. Animals per Group	**720. 5**	**721. 5**	**722. 5**	**723. 5**
4. Animals with haemangioma	**725. 2**	**726. 3**	**727. 4**	**728. 1**
9. Animals with haemangiosarcoma	**730. 5**	**731. 4**	**732. 6**	**733. 1 2**
4. Tumor bearing animals	**735. 6**	**736. 6**	**737. 9**	**738. 2**

Table 6.7.1.2 Incidence of female mice with haemangioma and/or haemangiosarcoma

Group:	**0 ppm**	**400 ppm**	**1200 ppm**	**4000 ppm**
Liver (No. Examined)	739.(1)	740.	741.(4)	742.(1
3. Haemangioma	744.		745. 2	746.5
7. Haemangiosarcoma	748.	749. .	750. 2	751.7
2. Bone Marrow (No. Examined)	753.(4)	754.	755.(2)	756.(2
7. Haemangioma	758.	759. -	760. 2	761.2
762. Haemangiosarcoma	763.3	764. 3	765. -	766.-
7. Ovary (No. Examined)	768.(1)	769.	770.(2)	771.(3
2. Haemangioma	773.	774. -	775. -	776.1
7. Haemangiosarcoma	778.	779. -	780. 1	781.-
2. Spleen (No. Examined)	783.	784. (1)	785. (1)	786.(
Haemangioma	787.	788. -	789. -	790.-
1. Haemangiosarcoma	792.5	793. 1	794. 1	795.4
6. Lymph Nodes (No. Examined)	797.(0)	798. (0)	799.(0)	800.(1
1. Haemangioma	802.-	803. -		804.-
5. Haemangiosarcorna	806.-	807. .	808. -	809.1
0. Uterus (No. Examined)	811.(0)	812. (0)	813.	814.
5. Haemangioma	816. -	817. -	818. 1	819. -
0. Haemangiosarcoma	821.-	822. -	823. -	824.-

5.	Spinal Cord (No. Examined)	826.(0)	827. (1	828.	829.
0.	Haemangioma	831. -	832. -	833. -	834. -
5.	Haemangiosarcoma	836.-	837. 1	838. -	839.-
0.	Sternum (No. Examined)j	841.(1)	842. (1)	843.(0)	844.(0
5.	Haemangioma	846.1	847. 1	848. -	849.-
0.	Haemangiosarcoma	851.-		852. -	853..
4.	Skin (No. Examined)	855.(2)	856. (1)	857.(0)	858.(0
9.	Haemangioma	860.2	861. -	862. .	863..
4.	Haemangiosarcoma	865.-	866. 1	867. -	868.-
9.	Limb (No. Examined)	870.(1)	871. (0)	872.(0)	873.(0
4.	Haemangioma	875.-	876. -		877.-
3.	Haemangiosarcoma	879.1	880. -	881. -	882.-
3.	Head (No. Examined)	884.(0)	885. (0)	886.(0)	887.(1
3.	Haemangioma		889. -	890. -	891.1
2.	Kidney (No. Examined)		893. 0	894.(0)	895.(1
6.	Haemangiosarcoma	897.-	898. -	899. -	900.1
1.	Muscle (No. Examined)	902.(0)	903. (P)	904.(0)	905.(1
6.	Haemangiosarcoma	907.-		908. -	909.1
0.	Subcutan. Tissue (No.	911.(1)	912. (0)	913.(0)	914.
5.	Haemangioma	916. 1	917. -	918. -	919. -
0.	Abdominal Cavity (No.	921.(1)	922. (0)	923.(0)	924.(0
5.	Haemangiosarcoma	926.1	927. -	928. -	929.-
0.	Mammary Gland (No.	931.(0)	932. (1)	933. (0)	934.(
5.	Haemangioma	936.-	937. 1	938. -	939.-
0.	**Animals per Group**	**941.55**	**942. 55**	**943.55**	**944.5**
5.	**Animals with haemangioma**	**946.6**	**947. 2**	**948. 5**	**949.8**
0.	**Animals with**	**951.6**	**952. 4**	**953. 4**	**954.1**
5.	**Tumor bearing animals**	**956.8**	**957. 5**	**958. 7**	**959.1**

The PWG finally concluded that was clear evidence of a treatment-related increase in incidence of animals with either haemangioma or haemangiosarcoma in the high dose (4000 ppm) of both sexes. This increase was largely due to the increased number of vascular tumors in the liver. In all other dose levels (400, 1200 ppm), there was no significant difference between the number of vascular tumor bearing animals and controls. The small difference in incidence of haemangiosarcomas in the liver between control and mid-dose males was considered a chance event because it did not attain statistical significance and approximates the historical control range of haemangiosarcomas of the liver. In thirteen studies conducted at the laboratory from 1985 to 1994, the range of haemangiosarcomas in male mice ranged from 1.8% to 18.3%, with an average of 9.16%. In females, the range of haemangiosarcomas ranged from 0% to 9.1%, with an average of 4.42% (reference: Ref 12, 2002b). The six haemangiosarcomas in the liver of the mid dose males constitutes a 10.9% incidence, which is within the range of historical controls. The PWG emphasized that the vascular tumors are in nature, multicentric and that it is most appropriate to consider the total number of animals with vascular neoplasms, rather than the individual organs with either primary or metastatic lesions. When the total number of tumor bearing animals is considered (see tables above) only the high dose group can be considered as significant in either sex. In addition, there was apparently an inverse relationship between hepatic and splenic vascular tumors. However, the number of splenic haemangiosarcomas decreased in the mid dose group in both sexes. Therefore, this organ-specific decrease was considered to be a spontaneous event, which underlined the use of total tumor-bearing animals as the appropriate data for analysis (Ref 82, 2002). The conclusion of the PWG was confirmed by an independent expert, which stated that the induction of haemangiosarcomas by PHMB in mice was not due to the chemical itself but rather the extreme toxicity with doses well in excess of the

MTD, which led to increased endothelial cell proliferation and ultimately development of the haemangiosarcomas. Administration of a dose that is approximately at the MTD had no effect on the incidences of haemangiosarcomas or other tumors. Finally, he concluded that PHMB can be considered as not carcinogenic in either rats or mice, specifically it does not induce haemangiosarcomas in these species at doses acceptable for long-term bioassays (Ref 22, 2009).

Following the above Pathology Working Group peer review of the vascular tumors observed in the two year life-time carcinogenicity study using C57Bl/10J/CD-1 Alpk mice), statistical analysis of the results was conducted by considering the incidence of animals with tumors at any site. Consequently, statistical analyses of the incidence of animals with haemangioma and haemangiosarcoma at any site were conducted allowing for time of death, using a prevalence analysis (assuming tumors were incidental), a death rate analysis (assuming the haemangiosarcomas were observed in a fatal context), and a combined analysis allowing for the observed context. Analyses were conducted both including and excluding the MTD exceeding top-dose. All tumor analyses were carried out separately for males and females.

Table 6.7.1.3 Main results of the intergroup comparison of individual tumor incidence of all groups and any tissues

	0 ppm	**400 ppm**	**1200 ppm**	**4000 ppm**
			960. Males	
61. Heamangioma	962. 2/55 (3.6%) p=0.003**	963. 3/55 (5.5%)	964. 4/55 (7.3%)	965. 11/55* (20%)
66. Heamangiosarcoma	967. 5/55 (9.1%) p=0.035*	968. 4/55 (7.3%)	969. 6/55 (10.9%)	970. 12/55 (21.8%)
71. Combined heamangioma/heamangiosarcoma	972. 6/55 (10.9%) p>0.001**	973. 6/55 (10.9%)	974. 9/55 (16.4%)	975. 20** (36.4%)
			976. Females	
77. Heamangioma	978. 6/55 (10.9%) p=0.357	979. 2/55 (3.6%)	980. 5/55 (9.1%)	981. 8/55 (14.5%)
82. Heamangiosarcoma	983. 6/55 (10.9%)	984. 4/55 (7.3%)	985. 4/55 (7.3%)	986. 10/55 (18.2%)

	p=0.247			
87. Combined heamangioma/heamangiosarcoma	988. 8/55 (14.5%) p=0.059	989. 5/55 (9.1%)	990. 7/55 (12.7%)	991. 15/55 (27.3%)

Thus, it was shown that when the MTD exceeding top-dose (4000 ppm) was excluded from any analyses, no statistically significant results were observed in either males or females for haemangioma, haemangiosarcomas or total incidence of vascular tumors combined. However, at 4000 ppm the incidence was increased in males and females but with statistically significance only in males (Ref 43, 2002).

More recently, there was a scientific advisory panel (SAP) review on PHMB covering carcinogenicity studies in rats and mice, pathology working group reviews, regulatory responses and mode of action studies (Ref 83, 2009). With regards to the above mice carcinogenicity study and based on the review of all available data it was concluded that:

- *PHMB shows no evidence of mutagenic activity.*
- *All group differences in vascular tumor incidence in rats and mice except those in the high-dose group in the mouse feeding study (4000 ppm) are incidental and therefore do not indicate carcinogenic activity.*
- *The incidence of vascular tumors in the high-dose group in the mouse feeding study compared with controls is not evidence of a carcinogenic alert since:*
    - *Dosing at 4000 ppm was well above the MTD which together with the mode of action analysis indicates they are not relevant to lower doses.*
    - *The difference in incidence of haemangiomas and haemangiosarcomas in mice in the 4000 ppm PHMB group compared with controls is modest.*
    - *These haemangiomas and haemangiosarcomas occur at an age where mice develop these tumors spontaneously. There is no evidence of their development occurring at an earlier age.*
    - *The tumors show no evidence of a shift to a less well-differentiated phenotype.*
    - *This pattern of a modest increase in incidence of vascular tumors in mice at two years, morphologically identical to those seen in controls, is similar to other agents that are considered non-carcinogenic. Notable examples include troglitazone and pregabalin which have been or are used as long-term therapy in humans.*
- *A plausible explanation has been advanced for the higher incidence of haemangioma and haemangiosarcoma in the mouse at the 4000 ppm dose group compared with controls. The data suggests that there is an underlying process of sustained cytotoxicity and increased DNA synthesis in hepatic endothelial cells in mice given high-doses of PHMB. As these effects do not occur at lower doses vascular tumors are unlikely to occur at low exposures. As a consequence the mouse liver tumor findings are irrelevant to use of PHMB as proposed where exposure to humans will be low.*
- *Haemangiomas and haemangiosarcomas found in man are biologically very different from those that occur in mice. In humans haemangiomas are common but bear no relationship*

to haemangiosarcomas. Haemangiosarcomas are rare and most of the known causes are dependent on genotoxic effects.

- *In man, angiogenesis and endothelial cell proliferation is well regulated and vascular proliferation as a result of prolonged injury is not associated with vascular tumor development.*

It is noteworthy to mention that the Committee for Risk Assessment (RAC; ECHA, Ref 32, 33 2011ab) after review of all data in their opinion and background document concluded that

> *PHMB increases the incidence of benign and malign vascular tumors in male and female mice by oral - and taking the lower strength of evidence due to MTD dosing into account - also by dermal route. The tumors are induced mainly in the liver, which is one of the target organs of PHMB and the increase is clearly seen at the high oral dose of 4000 ppm PHMB, which was reported to be above the MTD. However interpretation whether MTD was exceeded has uncertainties since the MTD was questioned in the light of high tumor-related mortalities and the assumption that reduced body weight gain could eventually be contributed to a hypoglycemic effect of PHMB. Dose-related increased incidences of vascular tumors were also observed at doses below the proposed MTD (mouse oral study at mid-dose). These increases are not interpreted to be incidental with regard to the dose-response relationship of vascular tumors at mid and high doses, the lower incidence or even absence in control groups, and some evidence for consistency across administration routes. They are considered biologically significant and attributed to treatment. […]*
>
> *RAC is aware that the overall evidence on carcinogenic potential of PHMB is not strong.*
>
> *With respect to PHMB the evidence of carcinogenicity (systemic and local) is mainly from a single experiment (mouse oral carcinogenicity study), but there is supporting evidence from other studies in mice […]. There are remaining uncertainties about interpretation with respect to the MTD (criteria (b) is valid).*
>
> *PHMB is not genotoxic in vitro and in vivo, but taking into account that the overall evidence on carcinogenicity is mainly on the evidence from one study in one species and no mode of action has been identified a classification as carcinogenic Carc 2 – H351 (CLP) and category 3; R40 (DSD) is warranted.*

There exists another carcinogenicity study in mice with low reliability due to the high fighting-related mortalities during the first 6 months and is presented only for the sake of completeness. Groups of 30 male and 60 female Swiss-derived albino mice were fed diets containing 0, 500, 1000 or 5000 ppm PHMB (Baquacil SB, batches: SDC/596 and ADGM 5911, 20% aqueous PHMB solution) equivalent to 0, 100, 200 and 1000 ppm of the active ingredient PHMB, for one week prior to pairing and during mating. Feeding continued for the females throughout pregnancy and lactation. All offspring were weaned at 3 weeks of age. At 5 weeks of age 50 males and 50 females were selected from each group. The offspring were fed the same diets as the parents throughout the experiment. After a further 80 weeks 10 males and 10 females per group were killed for pathological examination. The experiment was terminated when the overall mortality had reached 80%, 97 weeks after selection of offspring. Due to considerable evidence of fighting amongst male population, mortalities in the males were high during the first 6 months of the experiment especially for controls and animals receiving 100 or 1000 ppm PHMB. Mortalities in the females were generally low during the first 18 months of the experiments, and were lower on treated groups than on controls. The only effects associated with administration of the compound were a dose-related reduction in mean body weight gain in females and an increase in liver weights for males and females receiving 1000 ppm. However, food utilisation was poorer only at the 1000 ppm level and the slight body weight effect at 100 and 200 ppm was

considered to be a the result of a palatability problem. There was an increase of the absolute liver weight for males and females receiving 1000 ppm but there was no histopathological correlate. No treatment related increases in non-neoplastic or neoplastic findings were observed by histopathological and there was no evidence of a carcinogenic effect. Thus, the long-term exposure to PHMB was related to systemic toxicity, the NOEL was reported to be 200 ppm (corresponding to about 22 mg/kg bw/day) and there was no carcinogenic effect up to the highest dose level of 1000 ppm (corresponding to about 110 mg/kg bw/day; Ref 19, 1977b).

6.7.2 Dermal studies

80 Week Skin Painting Study in the mouse:

Study Design:

Reference:	Ref 18, 1977a
Date of report:	1977
Guideline/method:	Skin painting study prior to establishment of specific testing guidelines
Species/strain:	Mouse/Alpk:APfCD-1
Group size:	50 male and 50 female animals per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	SDC/596, ADGM 5911 (Baquacil SB, 20.0% aqueous PHMB solution)
Dose levels:	0, 0.6, 6.0, 30 mg/mouse/day (corresponding to about 0, 15, 150, 750 mg/kg bw/day)
Solvent:	Ethanol
Route:	Dermal (not-occluded)
Exposure period:	80 weeks
Exposure frequency:	5 day/week
GLP:	No
Published:	No

Material and methods:

PHMB (Baquacil SB, batches: SDC/596 and ADGM 5911, 20% aqueous PHMB solution) was examined for its possible carcinogenicity when applied to the skin of mice for a period of 80 weeks. Aqueous PHMB formulated in ethanol were applied to the shorn backs of groups of 50 male and 50 female Alpk:APfCD-1 mice at dose levels of 0, 0.6, 6.0 or 30 mg/mouse/day (corresponding to approximately 0, 15, 150 or 750 mg/kg bw/day), 5 days per week for 80 weeks. Shaving was repeated once weekly throughout the study period and care was taken to avoid lacerating the skin.

The animals were examined for mortality and clinical signs including behaviour, skin irritation, papillomas and skin tumors. Body weight and food consumption were determined at regular intervals. Routine visual examination of the eyes was part of the weekly detailed clinical examination. However, following the observation of exophthalmos in week 22, subsequently a more detailed ophthalmology was performed were in each 2 males and females of the control and mid dose groups and in each 5 animals per sex in the high dose group at weeks 32, 44, 64, and 73. Any animal which became moribund or distressed due to disease (including tumors) during the course of the experiment was killed and a full post-mortem examination was carried out. At termination all surviving animals were killed by an overdose of halothane and a comprehensive number of tissues and organs were processed, fixed, stained and examined by histopathology. Statistical analyses were performed by means of Student’s T-test and Chi square where appropriate.

Results:

The highest dose of 30 mg/mouse/day led to irritant effects on the skin and to a generally poor condition in the mice, which was accompanied by body weight loss and a high mortality incidence (76-78% of animals dying prior to study termination). There was only a transitory skin irritant effect on male mice receiving 6.0 mg/mouse/day and some reduction in body weight gain. There was no effect at 0.6 mg/mouse/day. Exophthalmos was noted in the eyes of the animals in the highest dose groups. However, this was not associated with histopathological changes in the thyroid, Harderian gland or the eye itself. Probably this has resulted from transfer of PHMB to the eye from the skin after grooming. No ocular effects were noted at the two lower dose levels. PHMB led to no carcinogenic effects on the skin. There was a variety of inflammatory hepatic changes in all groups including controls, but at 30 mg/mouse/day this was characterised by a severe hepatitis in some animals. These hepatic changes appeared to have been mainly responsible for causing increased numbers of deaths in the high dose level animals. Noteworthy to mention that the scientific advisory panel (SAP) mentioned above (Ref 83, 2009) assumed that the hepatitis may be related to infection with *Helicobacter hepaticus* and in general, Helicobacter infection might be associated with an increased incidence of hepatitis and hepatocellular neoplasms. There was a slight increase in the incidence of liver tumors observed at 30 mg/mouse/day (four in the control and ten at 30 mg/mouse/day). This was statistically significant only in the case of liver tumors of endothelial origin (both benign and malignant; two in the control and six at 30 mg/mouse/day), when the data were pooled over the whole study period and both sexes. There was no evidence of such an effect in animals receiving 0.6 or 6.0 mg/mouse/day. The long standing occurrence of the hepatitis in these mice was thought to be the reason for the slightly higher incidence of liver tumors in this top dose group. It is probable that the poor condition of these animals led to a greater susceptibility for infection involving the hepatic changes. No abnormal histopathological changes were noted at 0.6 or 6.0 mg/mouse/day.

Conclusion:

Under the conditions of this skin painting study for a period of 80 weeks in male and female mice there was no indication for a skin carcinogenic effect up to the highest dose level tested. The highest dose level was clearly above MTD due to excessive mortality and reduced body weight gain up to 50% in both sexes. The overall no effect level (NOEL) was 0.6 mg/mouse/day.

It is noteworthy to mention that the Committee for risk assessment (RAC; ECHA, Ref 33, 2011b) concluded: “There was no evidence of a carcinogenic effect of PHMB at dose levels up to 6.0 mg/mouse/day. The higher dose of 30 mg/mouse/day greatly exceeded the MTD. At this dose there was a slight increase in liver tumors consisting of hepatocellular adenoma in 4 animals versus 1 in the controls, haemangioendothelioma in 3 animals versus 1 in the controls, and angiosarcoma in 3 animals versus 1 in the controls. The incidence of females having vascular neoplasms at any site was statistically significantly for trend (US-EPA 2003). No compound-related histopathological changes were seen at 0.6 or 6.0 mg PHMB/mouse/day.

6.7.3 Overall conclusion on carcinogenicity studies

The carcinogenic potential of PHMB was sufficiently investigated in oral long-term studies in rats and mice as well as in a dermal skin painting study in mice.

The dietary administration of PHMB at dose levels of 0, 200, 600 and 2000 ppm in a combined chronic toxicity/carcinogenicity study in male and female rats for a period of up to two years led to treatment-related findings only at the high dose of 2000 ppm in the form slightly reduced survival in females, reduced body weight in both sexes and changes clinical-chemistry indicative for slight liver impairment. The criteria for a maximum tolerated dose (MTD) were achieved.

Although there is some controversial discussion, it can finally be concluded that there was no evidence of a carcinogenic effect associated with the administration of PHMB for two years at dose levels of up to 2000 ppm. The no observed effect level for chronic toxicity in the rat following dietary administration of PHMB for two years was 600 ppm (equivalent to about 36 and 45 mg/kg bw/day for males and females, respectively).

The dietary administration of PHMB in a life-time feeding study in mice at dose levels of 0, 400, 1200 and 4000 ppm led to marked toxicity at the highest dose level of 4000 ppm. This dose caused marked decreases in body weight gain and was clearly well in excess of a maximum tolerated dose. The mid-dose of 1200 ppm PHMB fulfilled the criteria for a maximum tolerated dose based on reduced body weight gain and non-neoplastic pathological changes at three sites (liver, recto-anal junction and gall bladder). The highest dietary concentration used, 4000 ppm, resulted in a markedly altered tumor profile. At this dose several squamous cell carcinomas of the recto-anal junction occurred in mice of both sexes and gall bladder papillomas occurred in two males. Vascular tumors, mainly haemangiosarcomas, were increased whereas lymphosarcomas were decreased in both sexes compared to the controls. Pituitary gland adenomas were also decreased in female mice. However the changes in tumor profile were considered to be of doubtful toxicological significance as the dose level exceeded clearly the MTD. 1200 ppm was the maximum tolerated dose and no observed effect level for oncogenicity. The results and assessment of the study author and study pathologist were highly controversial and many subsequent investigations in the form of additional statistically analyses, histopathological peer reviews and comprehensive data assessment of independent experts were performed. This discussion is still ongoing and no final conclusion was drawn until now. However, the majority of the experts expressed their opinion that based on the review of all data and a weight of evidence approach, that the observed incidence of vascular tumors in the high-dose group in the mouse feeding study compared with controls is not a carcinogenic alert. A plausible explanation has been advanced for the higher incidence of haemangioma and haemangiosarcoma in the mouse at the 4000 ppm dose group compared with controls. The data suggests that there is an underlying process of sustained cytotoxicity and increased DNA synthesis in hepatic endothelial cells in mice given high-doses of PHMB. As these effects do not occur at lower doses vascular tumors are unlikely to occur at low exposures. As a consequence the mouse liver tumor findings are irrelevant to use of PHMB as proposed where exposure to humans will be low.

However, the European Committee for Risk Assessment did not follow this conclusion and stated that the overall evidence on carcinogenic potential of PHMB is not strong but PHMB should be classified as carcinogenic Carc 2 – H351 (CLP) and category 3; R40 (DSD). In its minority opinion regarding the Carc. 2 classification (GHS) of PHMB an independent expert (Ref 46, 2011) concluded that:

> *Most likely the vascular tumors observed at high doses are the result of disturbed glucose metabolism with the consequence of impaired cellular energy supply and among other responses result in enhanced angiogenesis. Due to the anti-hypoglycaemic effect of PHMB such high exposure cannot occur in humans, so that the exposure conditions of the animal studies have no relevance to humans. Consequently, I cannot support the RAC proposal to classify PHMB as a Cat. 2 (GHS) carcinogen. Instead, no classification is warranted.*

Finally, five cancer studies have been carried out with PHMB in total (each two oral studies in rats and mice, one dermal study in mice). As no statistical analysis was carried out after tumor re-assessment of tumor types in the original study reports by the PWG, statistical analysis on the PWG tumor assessment for the two key studies in rats and mice (Ref 61, 1996; Ref 87, 1996) was performed (Ref 105, 2010; Ref 106, 2011).

The results from the statistical analysis on the rat study showed that no test was statistically significant at the 5% significance level (no p-value ≤0.05). The statistical test for a decreasing trend for haemangioma among the lymph node mesenteric neoplasms in male rats at the lowest three doses (0, 200, and 600 ppm) was statistically significant at the 6% significance level (p-value: 0.0577). This decrease in the vascular tumors observed in the mesenteric lymph nodes further reinforced the interpretation that the few non-statistically significant differences between groups occurred by chance and thus, were not related to PHMB treatment. In addition, the former rat study (reference: Ref 4, 1977) showed no increase in the number of vascular tumors at any site or an increase in total number of animals with vascular tumors at any dose at or below the MTD, which points to the variability between sites and studies. It has to be emphasized that interpretation of the mouse oral study should not include the top dose that excessively exceeded the MTD. Once this group was removed, no statistically significant vascular finding was observed in either the oral or dermal mouse studies, from pair wise comparisons or trend analysis.

Based upon independent expert analysis of the tumor data in the mouse and rat studies, the weight of evidence from five separate life-time cancer studies is reduced to a single dose above the MTD in the mouse oral study. However, findings at doses above the MTD are generally recognized as not relevant for the purposes of cancer classification (OECD, 2008). Thus, based on the weight of evidence of all data, PHMB should be considered to possess no carcinogenic potential for rats or mice.

Overall, with respect to the current and anticipated use conditions as preservative in cosmetic preparations up to a maximum concentration of 0.1%, the carcinogenic risk can be considered as negligible, if any and PHMB can be considered as safe for users of cosmetic products.

6.8 Reproduction toxicity

6.8.1 Fertility and reproduction

Study Design:

Reference:	Ref 86, 1995, Ref 84, 1993
Date of report:	1995
Guideline/method:	Comparable to OECD 416
Species/strain:	Rat/Alpk:APfSD (Wistar derived)
Group size:	26 males and 26 females per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	D4097 (Vantocil P, 20.2% aqueous PHMB solution)
Dose levels:	0, 200, 600, 2000 ppm (corresponding to about 0, 23 – 24, 70 – 71, 239 – 249 mg/kg bw/day in males, 0, 25 – 26, 77 – 79, 258 – 270 mg/kg bw/day in females)
Route:	Oral (diet)
Exposure period:	Two successive generations (F0 and F1 parental generation, F1 and F2 offspring)
GLP:	Yes
Published:	No

Material and methods:

PHMB (Vantocil P, batch: D4097, 20.2% aqueous PHMB solution) was investigated in a two-generation reproduction toxicity study for its effects on reproductive performance including

fertility as well as on postnatal development. The test substance was administered to groups of 26 male and 26 female weanling Alpk:APfSD (Wistar derived) rats (F0 parental generation) via the diet at concentration of 0, 200, 600 and 2000 ppm (corresponding to about 0, 23 – 24, 70 – 71, 239 – 249 mg/kg bw/day in males, 0, 25 – 26, 77 – 79, 258 – 270 mg/kg bw/day in females). After 10 weeks, the animals were mated and allowed to rear the ensuing F1 litters to weaning. The breeding programme was repeated with the F1 parental animals selected from the F1 offspring to produce the F2 litters after an 11 week pre-mating period. The test diets were fed continuously throughout the study. In the absence of a reliable method for quantitative analysis of PHMB in diet, the measurement of achieved concentration, homogeneity and stability in diet was not performed. However, the homogeneity of the mixing procedure was determined using an aqueous solution of a dyestuff and was performed at approximately 6 monthly intervals. Information demonstrating that the method used produced homogenous diets was reported separately (reference: Ref 84, 1993). The parental animals and offspring were examined for mortality and clinical signs including behaviour. Body weight (all animals) and food consumption (F0 and F1 parental animals) were determined at regular intervals. All parental animals and offsprings at scheduled kills were anaesthetized and killed by exsanguination. All parental animals which died or were killed intercurrently and at schedule were gross pathologically examined. The kidney, liver, testis and epididymis were weighed from all animals at termination. Selected organs and tissues with special emphasis of reproductive organs and grossly abnormal tissues were processed, fixed and stained and examined by histopathology. All pups surviving to termination and not selected for the next generation were killed on approximately day 29 post partum. Five male and five female F1 and ten male and ten female F2 pups from control and all treatment groups were scheduled to receive a full post mortem examination. The kidney, liver, testis and epididymis were weighed from all pups which received a full post-mortem at termination. The liver, kidney and grossly abnormal tissues were further processed, fixed, stained and examined by histopathology. Statistical analyses were performed by means of analysis of variance or covariance, Fisher’s exact test, Student’s T-test and/or double arcsine transformation of Freeman and Tukey.

Results:

PHMB binds to diet and no analytical method could be established. The ability of the mixing procedure to form a homogenous dietary preparation was demonstrated by means of a cationic dye and showed that the homogeneity was satisfactory.

Effects on parental animals:

The health status of the rats was generally maintained and in a good condition. In the parental F0 and F1 animals there was a decrease in body weight at 2000 ppm, which was occasionally accompanied by a small decrease in food consumption and reduced efficiency of food utilisation. This was seen throughout the whole study but was most marked in the F0 generation. There was no evidence of an effect of PHMB on any of the reproductive parameters assessed. The only unexpected finding was the high number of litter deaths in the F2 litter but this was clearly not related to PHMB as more litters were lost in the control and 200 ppm groups than in the 600 and 2000 ppm groups. Investigation of this finding indicated that the most probable cause was an environmental disturbance due to building work in an adjacent animal block. The litter losses did not affect the ability of the study to detect effects of PHMB on reproduction as a satisfactory number of F2 litters per group survived to weaning and provided information on the growth of offspring. No treatment related effect was noted by gross and histopathology in any group. There was some evidence of small increases in the kidney and liver weights of F0 males and F1 males and females receiving 2000 ppm PHMB. However, in the absence of related pathological

changes these were considered to be not biologically significant. The decreased weights of epididymides of F0 males were considered to be not related to treatment as there were no microscopic pathology findings and there was no similar effect in F1 males up to and including 2000 ppm.

Effects on the offspring:

There was no evidence of an effect of PHMB on any of the pup parameters measured including growth and development. There were some differences in the weights of liver and kidneys in F2 offspring at all dose levels. However differences in these organ weights were considered not to be related to the treatment as there was no evidence of a dose response and there were no supporting histopathological findings up to and including 2000 ppm.

Conclusion:

The dietary administration of microencapsulated PHMB in the two-generation reproduction toxicity study to groups of male and female rats at dose levels of 200, 600 and 2000 ppm group led to signs of systemic toxicity in the form of decreased body weights in animals of both sexes and both generations at 2000 ppm. There was no effect on reproductive performance and fertility as well as no effect on pre- or postnatal development. The NOAEL for systemic parental toxicity was 600 ppm (corresponding to about 70 – 71mg/kg bw/day in males, 77 – 79 mg/kg bw/day in females). The NOAEL for reproductive toxicity including fertility as well as for postnatal development was 2000 ppm (corresponding to about 239 – 249 mg/kg bw/day in males, 258 – 270 mg/kg bw/day in females).

In a previous three-generation reproduction toxicity study with lower reliability, PHMB (20% aqueous PHMB solution) was administered via the diet to groups of 10 male and 20 female Sprague-Dawley rats at concentrations of 0, 200, 600 and 1300 ppm. Ten to 12 week old animals were exposed for 9 weeks and then mated to produce F1a litters. After these litters had been weaned, a further 10 males and 20 females per group were selected to form the F1 parental generation. This process was repeated until the weaning of the F3A litter although half of this litter was delivered by Caesarean section for the study of potential teratogenic effects. Individual body weights, clinical condition and food consumption were recorded at regular intervals. During mating of the F2 parents, vaginal smears were examined. All pups delivered naturally were examined at birth and weaning and the number and total weight per sex recorded. At weaning, 10 male and 10 females F3A pups were necropsied and representative tissues stored for possible future histopathological examination. There were no indications of any adverse effect on body weight or food consumption in the F1 and P1 parental generations or on food consumption in the P2 generation. Evaluations of the various reproductive indices, sex ratios, and body weight data of the foetuses 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 were noted in the fetuses taken by Caesarean section. The dose level of 1300 ppm was the NOAEL general toxicity, reproductive function and fertility as well as for pre- and postnatal development (Ref 113, 1990).

6.8.2 Teratogenicity studies

6.8.2.1 Teratogenicity study in rats

Study Design:

Reference:	Ref 54, 1976
Date of report:	1976
Guideline/method:	Comparable to OECD 414
Species/strain:	Rat/Alderly Park strain
Group size:	20 - 22 pregnant rats per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	no data (Baquacil SB, 20% aqueous PHMB solution)
Dose levels:	0, 200, 1000, 2000 ppm (corresponding to about 0, 13, 54, 112 mg/kg bw/day)
Route:	Oral (diet)
Exposure period:	gestation days 0 – 20
Positive control:	Aspirin, 2900 ppm
GLP:	Yes
Published:	No

Material and methods:

PHMB (Baquacil SB, 20% aqueous PHMB solution) was tested for its prenatal developmental toxicity in Alderly Park rats. The test substance was admixed into the diet and administered to group of 20 – 22 pregnant rats at dietary dose levels of 0, 200, 1000 and 2000 ppm (corresponding to about 0, 13, 54, 112 mg/kg bw/day) on gestation days (GD) 0 – 20. A positive control group of 20 pregnant dams received 2900 ppm Aspirin in parallel. At terminal sacrifice 20 - 22 females/group had implantation sites and were considered as pregnant. The dams were examined for mortality, clinical signs and abortion. Body weight and food consumption were determined at regular intervals. On day 20 post coitum the animals were sacrificed and assessed by gross pathology. For each dam the number of implantation sites was determined. The fetuses were removed from the uterus, sexed, weighed and further investigated for external findings. Thereafter, nearly one half of the fetuses of each litter were examined for soft tissue findings according to Wilson technique and the remaining fetuses for skeletal findings after staining with Alizarin red. For statistical analysis the Student’s test or 2 x 2 contingency tables were employed.

Results:

Findings in the dams:

There were no mortality and no adverse clinical effects in any group. Maternal weight gain was significantly reduced in the groups receiving 1000 or 2000 ppm PHMB and the food consumption was also significantly reduced in these groups.

Reproduction data of dams:

Pregnancy was confirmed for 20 - 22 rats/group. Gestational parameters such as number of implantation sites, pre- and post-implantation losses were not influenced by the test substance at any dose level.

Examination of fetuses:

No dose-related effects were observed on fetal or litter weights. There was an increase in extra ribs at the high dose. However, this can be considered as indicative of fetal toxicity caused by maternal toxicity but not as a teratogenic effect. There was no further substance-related effect on fetal morphology including ossification of the skeleton in any of the treated groups.

Conclusion:

The oral administration of PHMB of 0, 200, 1000 and 2000 ppm (corresponding to about 0, 13, 54, 112 mg/kg bw/day) to pregnant rats during gestational days 0 – 20 resulted in maternal toxicity at 1000 and 2000 ppm in the form of reduced food consumption and body weight. Gestation was not affected at any dose group.Prenatal developmental toxicity in terms of a slight increase in fetuses with extra ribs was observed in the high dose groups only. However, this finding coincided with maternal toxicity at the same dose levels and was therefore considered as secondary. Thus, there was no indication of a selective effect on fetal morphology and especially no indication for teratogenicity. The NOAEL for maternal toxicity was 200 ppm (about 13 mg/kg bw/day) and for prenatal developmental toxicity was 1000 ppm (about 54 mg/kg bw).

6.8.2.2 Teratogenicity study in mice

Study Design:

Reference:	Ref 53, 1977, Ref 39, 40, 1981a, b
Date of report:	1977
Guideline/method:	Comparable to OECD 414
Species/strain:	Mouse/Alderly Park strain
Group size:	47 - 49 animals per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	no data (Baquacil SB, 20% aqueous PHMB solution)
Dose levels:	0, 10, 20, 40 mg/kg bw/day
Vehicle:	0.5% aqueous Tween 80 solution
Application volume:	10 mL/kg bw
Route:	Oral (gavage)
Exposure period:	gestation days 6 - 15
GLP:	Yes
Published:	No

Material and methods:

PHMB (Baquacil SB, 20% aqueous PHMB solution) was tested for its prenatal developmental toxicity in Alderly Park mice. The test substance was diluted with 0.5% aqueous Tween 80 solution and administered to group of 47 – 49 mice orally by gavage at dose levels of 10, 20 and 40 mg/kg bw/day on GDs 6 – 15. A standard dose volume of 10 ml/kg body weight was used for each group. The control group, consisting of 25 females, was dosed with the vehicle (0.5% aqueous Tween 80 solution) in parallel. At terminal sacrifice 21 - 28 females/group had implantation sites and were considered as pregnant. The dams were examined for mortality, clinical signs and abortion. Body weight and food consumption were determined at regular intervals. On day 18 post coitum all females were sacrificed and assessed by gross pathology. For each dam, the number of implantation sites was determined. The fetuses were removed from the uterus, sexed and weighed. Alternate fetuses from each litter were eviscerated and fixed in 70% methanol, the viscera being macroscopically examined for abnormalities. These fetuses were stained with Alizarin Red for subsequent skeletal examination. The remaining fetuses were preserved, decalcified in Bouin's fixative and sections were made through the head and thorax according to the technique of Wilson. The abdomen was examined by dissection, and sections made through the kidneys in order to examine their internal structure. Twenty litters per group were examined in detail and the remaining fetuses preserved. For statistical analysis the Student’s test or 2 x 2 contingency tables were employed.

Results:

Findings in the dams:

There was no mortality and no substance-related adverse clinical signs. Food consumption was not affected but the mean body weight gain at 40 mg/kg bw/day was slightly but not statistically significant reduced.

Reproduction data of dams:

Pregnancy was confirmed for 21 – 28 dams/group and thus, was very low in all groups including the control. The mean pregnancy rate over all groups was 59%, whereas the expected value was about 80%. Gestational parameters such as implantation sites, pre- and post implantation loss, litter size and weight, resorption were not influenced by the test substance at any dose level.

Examination of fetuses:

Fetal weight and sex distribution was not affected by treatment. There was no effect on external, soft tissue or skeletal fetal morphology with the exception of a marginal retarded ossification of primarily the forelimb and hindlimb digits and numbers of caudal vertebrae at 20 and 40 mg/kg bw/day. The incidence of wide fontanels and poorly ossified frontal bones in the skull at these dose levels indicated also retarded ossification.

Conclusion:

The oral administration of PHMB at dose levels of 10, 20 and 40 mg/kg bw/day to mice from implantation to day 15 of gestation resulted in marginal signs of maternal toxicity in the form of a slightly but not statistically significant reduction in mean body weight gain at the 40 mg/kg bw/day only. Gestation was not substance-related affected and litter and fetal parameters i.e. numbers of implantations, litter size, numbers of resorption, fetal weight and litter weight were not affected by treatment. There was no increase in the incidence of fetal abnormalities. However, there was a marginal retardation of ossification in single skeletal structures at 20 mg/kg bw/day and above. Under the conditions of this study, the NOAEL for maternal toxicity was 40 mg/kg bw and for prenatal developmental toxicity was 10 mg/kg bw/day. However, the overall validity of the study can be considered as limited due to the uncommonly very low pregnancy rate in all groups including the control. In addition, the limitation of the examination of only 20 and not all possible litters may have also biased the examinations.

6.8.2.3 Teratogenicity study in rabbits

Study Design:

Reference:	Ref 9, 1993b
Date of report:	1993
Guideline/method:	Comparable to OECD 414
Species/strain:	Rabbit/New Zealand white
Group size:	20 time-mated animals per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	D4097 (Vantocil P, 20.2% aqueous PHMB solution)
Dose levels:	0, 10, 20, 40 mg/kg bw/day
Vehicle:	Deionised water
Route:	Oral (gavage)
Exposure period:	gestation days 8 - 20

GLP: Yes
Published: No

Material and methods:

PHMB (Vantocil P, batch: D4097, 20.2% aqueous PHMB solution) was tested for its prenatal developmental toxicity in New Zealand white rabbits.. The test substance was diluted with deionised water and administered to group of 20 time-mated rabbits orally by gavage at dose levels of 10, 20 and 40 mg/kg bw/day on gestational days 8 – 20. The control group, consisting of 20 females, was dosed with the vehicle (deionised water) in parallel. The stability and correctness of dose levels were analytically examined. The dams were examined for mortality, clinical signs and abortion. Body weight and food consumption was determined at regular intervals. On gestation day 30 the animals were killed by an intravenous injection of pentobarbitone sodium solution and a macroscopic examination was performed. The uterus from any animal without clear evidence of implantation was removed and stained with ammonium polysulphide to determine whether or not implantation had occurred. For pregnant animals the intact gravid uterus was weighed and the number of corpora lutea, number and position of implantations, live fetuses, intra-uterine deaths and fetal weights were determined. The fetuses were killed with an intracardiac injection of pentobarbitone sodium solution. Each fetus was examined for external abnormalities and cleft palate. All fetuses were then examined internally for visceral abnormalities, sexed, eviscerated and fixed in methanol. The head of each foetus was cut and the brain was examined. The carcasses were further processed and staining with Alizarin Red S for skeletal abnormalities. An analysis of variance was performed followed by Fisher's exact test or double arcsine transformation according to Freeman and Tukey.

Results:

The achieved concentrations of PHMB were within 7% of the target concentrations and the stability of PHMB in water was analytically verified for at least 21 days at ambient temperature.

Findings in the dams:

In high dose animals there were 6 intercurrent deaths. One animal was killed in extremis on day 22 due to inappetence and weight loss present from day 11. Another animal was found dead on day 20 but blood was present in the thoracic cavity suggesting a possible mis-dosing. The other 4 deaths in the 40 mg/kg bw/day group were all due to abortion and in all of these cases, inappetence and weight loss were present during the dosing and post-dosing periods. At 40 mg/kg bw/day the animals, which were killed or died intercurrently, losses body weight, while the surviving animals showed retarded body weight gains and the food consumption was reduced. The animals killed intercurrently showed a variety of abnormalities in the stomach or cecum at necropsy consistent with irritation and inflammation at site of contact.

Reproduction data of dams:

At 40 mg/kg bw/day there was one total resorption and only two foetuses in the litter and the dam showed some weight loss during the dosing period. Gestational parameters such as number of corpora lutea, implantation sites and preimplantation loss were not influenced by the test substance at any dose level. Sex distribution was not influenced by the test substance.

Examination of fetuses:

Signs of prenatal developmental toxicity were not noted at any dose level. The incidence of fetuses with minor or major external/visceral defects was similar for all groups including the control group and none of the specific defects provided evidence for an adverse effect. The

incidence of fetuses with minor skeletal defects was similar for the control, 10 and 40 mg/kg bw/day groups. At 40 mg/kg bw/day, the percentage of fetuses with unossified 5th sternebrae or with fused 4th and 5th sternebrae was increased, but in the absence of changes in fetal development associated with PHMB, this finding was considered as unlikely to be substance-related.

Conclusion:

The oral administration of PHMB at dose levels of 0, 10, 20, 40 mg/kg bw/day to pregnant New Zealand white rabbits from implantation to day 20 of gestation resulted in overt maternal toxicity at 40 mg/kg bw/day in the form of intercurrent deaths, reduced food consumption, body weight loss during treatment and one total resorption. Abortion was the reason for termination of four animals but this is considered to be secondary to maternal toxicity, rather than a direct effect of PHMB, since there was no increase in post-implantation lass (indicative of an effect on foetal survival) amongst animals with live foetuses at termination. There was no effect of PHMB on the number, growth or survival of the fetuses and there was no evidence for teratogenicity. There was no adverse effect of administration of PHMB on the incidence of variants or on the ossification at all dose groups. There were no clear treatment related increases in the incidence of minor defects. The incidence of sternebral anomalies was increased in fetuses at 40 mg/kg bw/day but, in the absence of changes in fetal development associated with PHMB, this finding can be considered as unlikely to be substance-related. The NOAEL for maternal toxicity was 20 mg/kg bw/day and for prenatal developmental toxicity was 40 mg/kg bw/day.

Previously to the above mentioned study, two range-finding studies were carried out. In the first range-finding study two non pregnant female New Zealand white rabbits received PHMB orally by gavage at a dose level of 40 mg/kg bw/day from days 1 - 3 and 80 mg/kg bw/day from day 4 until termination on day 12. Body weights, clinical observations and food consumption were recorded daily. At the end of the study, animals were humanely killed by intravenous administration of an overdose of pentobarbitone sodium and were subjected to a limited macroscopic post mortem examination. At 40 mg/kg bw/day there were no significant clinical observations or body weight losses. Within 1 - 2 days of increasing the dose to 80 mg/kg bw/day both rabbits showed clear signs of toxicity: inappetence and an associated gradual weight loss, subdued behaviour and an ungroomed/dirty appearance. On day 12 of the study one rabbit was found dead and the other humanely killed. Post mortem examination revealed evidence of gastric irritation/ulceration as well as caecal and jejunal distension and abnormal colonic contents in the rabbit that was found dead. There were no significant macroscopic abnormalities in the other rabbit. Based on these results, 80 mg/kg bw/day was considered to be a maximal top dose level for the subsequent dose range finding study in the pregnant rabbit (Ref 7, 1992).

Subsequently, groups of 10 time-mated female New Zealand White rabbits were dosed by gavage with PHMB at dose levels of 0, 40, 60 or 80 mg/kg bw/day in deionised water. The control group received deionised water only. The rabbits were dosed on days 8 to 20 (inclusive) of gestation. On Day 30 of gestation the rabbits were killed and their uteri examined for live fetuses and intra-uterine deaths. The fetuses were weighed, examined for external and visceral abnormalities and then discarded. Administration of PHMB to pregnant rabbits caused overt toxicity at 60 and 80 mg/kg bw/day, resulting in termination of affected animals during the dosing or post-dosing period. The clinical signs were non-specific (inappetence, weight loss and subdued behaviour) and probably secondary to the gastric irritation present in most of these animals. A no-observed effect level for maternal toxicity was not established since two rabbits showed similar signs of toxicity at 40 mg/kg bw/day. In animals surviving to scheduled termination, there was no evidence for an adverse effect of PHMB on the number or survival of

the offspring. A small reduction in fetal weight, indicative of slight fetotoxicity, was present at 60 mg/kg bw/day and was probably secondary to the maternal toxicity present at this dose level. The no observed effect level for fetotoxicity was 40 mg/kg bw/day. On the basis of these results, dose levels of 10, 20 and 40 mg/kg bw/day were selected in the subsequent main prenatal developmental toxicity study reported above (Ref 8, 1993a).

In addition, there exists a further old prenatal developmental toxicity study in rabbits with low reliability. Groups of 14 – 15 inseminated New Zealand white rabbits received PHMB (20% aqueous solution) at dose levels of 0, 10, 40 and 160 mg/kg bw/day orally by gavage during gestation days 6 – 18. The body weights were recorded and all animals were observed for clinical findings. On day 29 of gestation, the dams were euthanized and the uterine were examined. The fetuses were examined for soft tissue and skeletal findings. The administration of 10 and 40 mg/kg bw/day had no effect on pregnancy or on maternal and fetal survival. The number of soft tissue or skeletal abnormalities did not differ from the number in the control group at these dose levels. At 160 mg/kg bw/day maternal toxicity was reported which led to impaired implantation, increased early fetotoxicity and increased resorbed and dead fetuses (Ref 3, 1976).

6.8.3 Overall conclusion on reproductive toxicity

In a two-generation reproduction study, the dietary administration of PHMB to groups of male and female rats at dose levels of 200, 600 and 2000 ppm group led to signs of systemic toxicity in the form of decreased body weights in animals of both sexes and both generations at 2000 ppm. There was no effect on reproductive performance and fertility as well as no effect on pre- or postnatal development. The NOAEL for systemic parental toxicity was 600 ppm (corresponding to about 70 – 71mg/kg bw/day in males, 77 – 79 mg/kg bw/day in females). The NOAEL for reproductive toxicity including fertility as well as for postnatal development was 2000 ppm (corresponding to about 239 – 249 mg/kg bw/day in males, 258 – 270 mg/kg bw/day in females).

In prenatal developmental toxicity studies, no selective effects on fetal morphology were observed in rats, mice and rabbits. The oral administration of PHMB of 0, 200, 1000 and 2000 ppm (corresponding to about 0, 13, 54, 112 mg/kg bw/day) to pregnant rats during gestational days 0 – 20 resulted in maternal toxicity at 1000 and 2000 ppm in the form of reduced food consumption and body weight. Gestation was not affected at any dose group. Prenatal developmental toxicity in terms of a slight increase in fetuses with extra ribs was observed in the high dose groups only. However, this finding coincided with maternal toxicity at the same dose levels and was therefore considered as secondary in nature. Thus, there was no indication of a selective effect on fetal morphology and especially no indication for teratogenicity. The NOAEL for maternal toxicity was 200 ppm (about 13 mg/kg bw/day) and for prenatal developmental toxicity was 1000 ppm (about 54 mg/kg bw). The oral administration of PHMB at dose levels of 10, 20 and 40 mg/kg bw/day to mice from implantation to day 15 of gestation resulted in marginal signs of maternal toxicity in the form of a slightly but not statistically significant reduction in mean body weight gain at the 40 mg/kg bw/day only. Gestation was not substance-related affected and litter and fetal parameters i.e. numbers of implantations, litter size, numbers of resorption, fetal weight and litter weight were not affected by treatment. There was no increase in the incidence of fetal abnormalities. However, there was a marginal retardation of ossification in single skeletal structures at 20 mg/kg bw/day and above. The NOAEL for maternal toxicity was 40 mg/kg bw and for prenatal developmental toxicity was 10

mg/kg bw/day. However, the overall validity of the study is considered to be limited due to the uncommonly very low pregnancy rate in all groups including the control. In addition, the limitation of the examination of only 20 and not all possible litters may have also biased the examinations. The oral administration of PHMB at dose levels of 0, 10, 20, 40 mg/kg bw/day to pregnant New Zealand white rabbits from implantation to day 20 of gestation resulted in overt maternal toxicity at 40 mg/kg bw/day in the form of intercurrent deaths, reduced food consumption, body weight loss during treatment and one total resorption. There was no effect of PHMB on the number, growth or survival of the fetuses and there was no evidence for a selective effect on fetal morphology including teratogenicity. The NOAEL for maternal toxicity was 20 mg/kg bw/day and for prenatal developmental toxicity was 40 mg/kg bw/day.

6.9 Toxicokinetics and metabolism

6.9.1 Toxicokinetics

Absorption, distribution, metabolism and excretion in rats

Study Design:

Reference:	Ref 80, 1995a
Date of report:	1995
Guideline/method:	Absorption, distribution, metabolism and excretion screening
Species/strain:	Rat/ Alpk:APfSD (Wistar derived)
Group size:	3 – 5 males and 3 – 5 females per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	a) non-labelled: no batch stated (20% aqueous PHMB solution) b) labelled: no batch stated, [^{14}C]-labelled PHMB, specific activity: 1.85 GBq/4mmol (hexamethylene diamine)
Dose levels:	20 mg/kg bw
Vehicle:	sterilised, double deionised water
Route:	Oral (gavage)
Exposure:	Single application
GLP:	Yes
Published:	No

Material and methods:

Absorption, distribution, metabolism and excretion of PHMB were investigated in Alpk:APfSD (Wistar derive) rats. Non-labelled PHMB as 20% aqueous solution and [^{14}C]-labelled PHMB (specific activity: 1.85 GBq/4mmol) was investigated and were fractionated into low, medium and high molecular fractions (MWtF) prior to use. The study was conducted in three phases to examine biliary excretion, bioavailability as well as excretion and tissue retention. In the biliary excretion experiment, three male and three female bile duct cannulated rats received each a single oral dose of 20 mg [^{14}C]-PHMB/kg bw. The urinary, biliary and faecal excretion of radioactivity was monitored for two days after dosing. In the bioavailability part, three groups of four male rats received each a single oral dose of a low, medium or high molecular weight fraction of [^{14}C]-PHMB at a dose level of 20 mg/kg bw. Urinary and faecal excretion of radioactivity was monitored for three days after dosing.

The excretion and tissue retention of the low molecular weight fraction were examined in each five male and five female rats, which were dosed with a single oral dose of 20 mg [^{14}C]-low molecular weight fraction PHMB/kg bw. The urinary and faecal excretion of radioactivity was monitored for three days after dosing, at which time the residual radioactivity was measured in blood, selected tissues and in the residual carcasses. Forty eight (biliary excretion) or 72 hours (bioavailability, excretion, retention) after dosing the rats were anesthetized and killed by exsanguination, blood were collected, the gastro-intestinal tract was excised, washed and retained with the residual carcass for analysis. In addition, the brain, liver, gonads, lungs, heart, spleen and kidneys as well as samples of fat, femur and femoral muscle were retained for analysis of radioactivity by liquid scintillation counting.

Results:

Biliary excretion:

No sex difference was observed in excretion profiles with over 96% of the administered dose excreted in faeces, less than 3% in urine and less than 0.2% was eliminated in bile.

Bioavailability:

For all three fractions more than 94% was excreted in faeces. Rats dosed with low, mid and high molecular weight fractions of PHMB excreted 5.2%, 0.2% and 0.2% in urine respectively.

Excretion and Tissue Retention of low molecular weight fraction:

More than 93% of the dose was excreted in the faeces of male and female rats. A sex difference was observed in urinary excretion with males eliminating 7.8% of the dose in urine compared with 2.6% by females. Seventy two hours after dosing, the highest concentrations of radioactivity in the tissues analysed were present in the liver and kidneys of males (0.57 and 0.504 equiv./g respectively) and females (0.75 and 0.8111g equiv./g respectively). The residual carcasses contained 0.22% of the dose for males and 0.28% for females. The total mean percentage recoveries of the administered radioactivity, including excreta and tissue residues, amounted to 102.8% for male rats and 97.0% for females. Chromatographic analysis of male and female urine samples showed that urinary radioactivity co-chromatographed with the lower molecular weight components of PHMB. Thus, apparently, only the lower molecular weight components of PHMB were absorbed. The bulk of each dose remained unabsorbed and was excreted fairly rapidly in faeces, from which the radioactivity was not readily extractable.

Conclusion:

Following the single oral administration of an aqueous solution of 20 mg [^{14}C]-PHMB/kg bw, PHMB was poorly absorbed in the rat. Three days after administration of a single dose in water 0.2 to 7.8% of PHMB was excreted in the urine and 0.2 to 1.3% was detected in the carcass with the liver and kidneys showing the highest concentrations. In general, the highest oral absorption and urinary excretion are observed, when animals received the low molecular fraction of PHMB. In general, the highest oral absorption and urinary excretion are observed, when animals received the low molecular fraction of PHMB. In cannulated rats only 0.2% of administered PHMB was excreted in bile. Finally, the vast majority of the administered PHMB remained unabsorbed and was eliminated in the faeces under the conditions of this study.

There exists a previously performed explorative screening study on the possible absorption of PHMB after oral administration with low reliability. Ten NMRI mice received a single oral application of 2.0 mL radiolabelled PHMB ([C^{14}]-Vantocil, no further information) by gavage. The mice were deeply anesthetized and frozen in acetone at -70 °C at 2, 6, 18, 24 and 48 hours

after administration. The mice were further processed and 20 µ whole-body sections were cut on a microtome. The sections were collected, freeze-dried and fixed on a x-ray film for one months. The analysis showed that only the gastro-intestinal tract contained any radioactivity. Thus, under the conditions of this screening study whole body autoradiography showed that no appreciate absorption of PHMB took place in mice after single oral application (Ref 70, 1976b).

Oral bioavailability study in rats

Study Design:

Reference:	Ref 79, 1995b
Date of report:	1995
Guideline/method:	Bioavailability screening
Species/strain:	Rat/ Alpk:APfSD (Wistar derived)
Group size:	5 males and 5 females per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	a) non-labelled: no batch stated (20% aqueous PHMB solution) b) labelled: no batch stated, [^{14}C]-labelled PHMB, specific activity: 1.85 GBq/4mmol (hexamethylene diamine)
Dose levels:	200, 20000 ppm
Route:	Oral (diet)
Exposure:	14 days
GLP:	Yes
Published:	No

Material and methods:

The bioavailability of PHMB following dietary administration was studied in groups of five male and five female Alpk:APfSD rats. The animals were fed diets containing either 200 ppm or 2000 ppm unlabelled PHMB, corresponding to the lowest and highest dose levels in the combined chronic toxicity/carcinogenicity study, for a period of fourteen days. At the end of this period two groups of five animals of each sex received a single oral dose of diet, incorporating [^{14}C]-PHMB, as a 9% suspension in the dose vehicle (4 mL/kg bw). The high dose corresponded to 0.8 mg [^{14}C]-PHMB/kg bw (2 MBq/kg bw) and the low dose to 0.08 mg [^{14}C]-PHMB/kg bw (0.2 MBq/kg bw). Thereafter, the animals were housed in metabolism cages and excreta (urine, faeces) were collected for 72 hours following the radiolabelled dose.

Results:

The pattern of absorption and excretion following dietary administration of [^{14}C]-PHMB was similar to that previously observed following a single oral dose of aqueous [^{14}C]-PHMB (see above, reference: Ref 90, 1995a). Faeces were the principal route of excretion of radioactivity. At the 200 ppm dose level, 105% and 109% and at 2000 ppm, 106% and 105% of the administered doses for male and female rats were detected, respectively. At the 2000 ppm dose level the corresponding percentages were 106% and 105% respectively. Urinary excretion was much lower with 2.1% and 2.2% or 2.3% and 1.8% being excreted by male and female rats at 200 and 2000 ppm, respectively. Small amounts of the administered radioactivity were found in the contents of the gastrointestinal tract at both dose levels (less than 1%). Similar amounts of radioactivity were analysed in the tissues of the gastro-intestinal tract (0.02% - 0.1%). At 200 ppm only 1.3% and 1.1% of the applied dose was detected in the carcass of males and females, while at 2000 ppm only 0.2% could be found in the carcass of both sexes. The levels of radioactivity found in blood were similar for both sexes at both dose levels, ranging between

0.001 and 0.003 µg equivalent/g. Plasma levels were lower than those in whole blood, being lower than the limit of detection in all samples. The total mean percentage recoveries, including excreta and tissue residues, were 110.6% and 114.3% at 200 ppm and 109.4% and 107.4% at 2000 ppm for males and females, respectively.

Conclusion:

The bioavailability screening in rats, with repeated dietary administration of PHMB at 200 and 2000 ppm for 14 days followed by a single oral administration of an aqueous [^{14}C]-PHMB solution, showed that about 4.7% and 3.9% were absorbed at 200 ppm and 3.0% and 2.6% at 2000 ppm by male and female rats, respectively. Finally, the vast majority of the administered PHMB remained unabsorbed and was eliminated in the faeces under the conditions of this study.

In an older study with lower reliability, the gastro-intestinal absorption of PHMB was studied in adult male Alderly Park (Wistar derived) rats. A group of five animals received a single oral dose of 20 mg [^{14}C]-PHMB/kg bw by gavage and the animals were kept singly for 10 days to examine excretion. Other groups of each 3 male animals received a diet containing 20 ppm [^{14}C]-PHMB for up to 5 weeks. Five groups were killed at one week intervals and different tissues were collected to determine distribution. Thereafter, the remaining 3 groups remained untreated and were killed at intervals of 1, 3 and 5 weeks. For the urinary excretion investigation, 24-hour urine was collected from 5 rats, which were dosed once with 200 mg [^{14}C]-PHMB/kg bw by gavage. The results showed that the gastro-intestinal absorption in rats was only 5.6% of the single oral dose, and after dietary administration for up to 5 weeks, the tissue concentrations did not exceed 0.3 µg/g. Thus, it was shown by simple chromatographic and spectroscopic methods that the urinary polymer-related material consisted of small amounts of PHMB-oligomers, with two cyanoguanidino end groups, as well as the trace constituents, 3,3-dicyano-1,1-hexamethylenediguanidine and a compound, which was considered to be 1-(6-aminohexyl)-3-cyanoguanidine that is formed during the synthesis of PHMB. The authors concluded finally, that the constituents of PHMB were not metabolized in rats under the conditions of this study (Ref 10, 1975).

6.9.2 Overall conclusion on toxicokinetics and metabolism

Finally, no valid data on oral absorption are available, but screening studies in rats indicated that PHMB is poorly absorbed by the oral route. The vast majority of PHMB remained unabsorbed and was excreted mainly via the faeces.

From an oral (gavage) single dose toxicokinetic study using different molecular weight fractions of PHMB, up to 8.5 % of the applied radioactivity might be considered bioavailable (sum of urinary excretion and radioactivity in tissues and residual carcass at study termination) which might be rounded to 10 %.

6.10 Human data

6.10.1 Irritation

Human insult patch test (HIPT)

Study Design:

Reference:	Ref 107, 2001
Date of report:	2001
Guideline/Method:	Insult patch test according to standards set by Japanese patch test study group.
Species:	Human volunteers
Group size:	45 volunteers (17 males, 28 females)
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	No. 0237 (Cosmocil CQ, 20% aqueous PHMB solution)
Vehicle and control:	Purified water
Route:	Topical application to the medial surface of the upper arm
Exposure:	24 h.
Concentrations:	1.5, 3.0, 5.0% aqueous formulation (corresponding to 0.3, 0.6, 1.5% PHMB)
Scoring:	30 min and 24 h after patch removal Scale with grades 0 – 4 (no reaction – vesicles, erosions, necrosis)
GLP:	Yes
Published:	No

Material and methods:

The skin irritation property of PHMB (batch: 0237, Cosmocil CQ, 20% aqueous PHMB solution) was examined in a Human insult patch test. The study was performed in 45 consenting healthy volunteers (17 males and 28 females). The patches (Finn-chamber) containing test concentrations of 1.5, 3.0, 5.0% of the aqueous formulation (corresponding to 0.3, 0.6, 1.5% PHMB) were applied to the skin of the medial part of the arm. For control purposes, the vehicle (purified water) was examined in parallel. The plaster was removed after 24 hours and the skin reactions were examined 30 minutes after removal (observation at 24 hours). The skin was again examined 24 hours after removal (observation at 48 hours) and the skin reactions were graded by a scale with grades 0 – 4 (no reaction – vesicles, erosions, necrosis) to calculate the irritation index according to the "Standards set by the Japanese Patch Test Study Group". The skin irritation properties of the test solutions were evaluated according to the criteria of skin irritation index

Results

Forty-five volunteers completed the study. The application of purified water caused skin reactions at 24 h reading and the skin irritation index was calculated as 6.6. No skin reaction was noted at the 48 h reading. The application of the 1.5% and 3.0% aqueous formulations led to skin reactions at 24 h readings and the skin irritation index was calculated as 5.5 each. No skin reaction was noted at the 48 h reading. The application of the 5.0% aqueous formulations led to skin reactions at 24 h and 48 readings and the skin irritation index was calculated as 8.8.

Conclusion

The application of aqueous PHMB solutions to healthy human volunteers for 24 hours led to skin reactions comparable intensity and duration to those observed with the vehicle. The authors concluded therefore that the aqueous PHMB solutions up to the concentration of 5.0% (corresponding to 1.5% PHMB) showed no undesirable primary skin irritation property in humans.

6.10.2 Sensitization

Study Design:

Reference:	Ref 104, 1981
Date of report:	1981
Guideline/Method:	Repeated insult patch test according to the method of Shelanski, 1951, Shelanski and Shelanski, 1953 and Stotts, 1980
Species:	Human volunteers (males and females)
Group size:	Total panel: 209 volunteers enrolled (104 males, 105 females, age range: 18 – 64)
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	no data (Vantocil IB, 20% aqueous PHMB solution)
Vehicle:	Distilled water
Route:	Topical application to the dorsal surface of the upper arm
Induction phase:	Application of the test material three times per week (e.g., Monday, Wednesday, Friday) for a total of ten applications.
Challenge phase:	About 2 weeks after final induction application following the same procedure as for induction
Concentrations:	a) Preliminary panel (54 volunteers enrolled/49 completed: Induction: 2% throughout except 2 or 3 patches at 4% Challenge: 0.1%, 0.5%, 1%, 2% b) Main panel (126 volunteers enrolled/114 completed) Induction: 2% throughout except 1 or 2 patches at 4% Challenge: 0.05%, 0.1%, 0.2%, 0.5% c) Additional panel (29 volunteers enrolled/28 completed) Induction: 4 or 5 patches at 2%, 5 or 6 patches with distilled water Challenge: 0.05%, 0.1%, 0.2%, 0.5%
Scoring:	Induction: prior to next application Challenge: 48 and 96 h after application Scale with grades 0 – 5 (no clearly visible reaction – strong reaction spreading well outside the test side, bullous reaction)
GLP:	Yes
Published:	No

Material and methods:

PHMB (Vantocil IB, 20% aqueous PHMB solution) was tested for its potential to induce primary or cumulative irritation and/or allergic contact sensitization in a repeated insult patch test, when applied to healthy human volunteers. For the study 209 volunteers (104 males, 105 females, age range: 18 – 64) were enrolled and 191 volunteers completed the study. In principle, 0.5 mL aliquot of PHMB was applied a 4 cm² Webril swatch affixed to adhesive square tape, which was then applied to the upper arm for 24 hours under occlusion. Ten 24 hour induction applications were followed two weeks later by a 24-hour occluded challenge application. A preliminary panel of 54 subjects was exposed during induction at 2% PHMB for the 6 first patches. Due to low level of irritation, exposure level was increased to 4% for the 3 next patches. On the appearance of symptoms of skin sensitisation in a number of subjects, the concentration was reduced to 2% for the remainder of the induction patches of this panel. At challenge the subjects received concentrations of 0.1%, 0.5%, 1% and 2% for 24 h under occlusion. In the main panel 126 subjects were exposed to 4% PHMB during induction as a first result of the irritation level from the preliminary panel. As the number of cases of reactions increased in the preliminary panel, the concentration was decreased to

2% from the 4th patch until the end of the induction phase. At challenge the subjects received concentrations of 0.05%, 0.1%, 0.2% and 0.5% for 24 h under occlusion. In an additional panel 29 subjects were exposed to 2% PHMB during induction for 5 patch exposures before the results of the preliminary panel indicated this was a sensitising level. Therefore the remaining patches were applied as distilled water applications to avoid unnecessary risk. The concentrations chosen for challenge were 0.5%, 0.2%, 0.1% and 0.05%. The skin reactions were scored during the induction period prior to the next application and during the challenge period at 48 and 96 h after application on a scale with grades 0 – 5 (no clearly visible reaction – strong reaction spreading well outside the test side, bullous reaction).

Results:

In the preliminary panel 49 subjects completed the study. At challenge, 8 subjects out 49 (16%) elicited skin reactions at 2% PHMB, 7 out of 49 (14%) at 1% and 0.5% PHMB and 2 (4%) subjects showed weak reactions at 0.1% PHMB. In the main panel 114 subjects completed the study. At challenge, 18 subjects out 114 (16%) elicited skin reactions at 0.5% PHMB, 7 out of 114 (6%) at 0.2% PHMB and none at 0.1% and 0.05% PHMB. The intensity of the reactions was generally lower than that observed from the preliminary panel. Two other panellists had reactions, which appeared during the rest period as a result of the 2% induction but were negative to the four lower challenge concentrations. Ten other subjects revealed indications of weak sensitisation during late induction to 2% and showed no reactions at challenge to the four lower concentrations. In the additional panel 28 subjects completed the study. At challenge, one subject out of 18 reacted to the high level of 0.5% only. All other subjects showed no skin reactions.

Conclusion

The human repeat insult patch test in male and female volunteers showed that PHMB is capable of causing skin sensitisation, after repeated occlusive exposure at concentrations of at least 2% PHMB. In addition, there is information available that in over 30 years of manufacturing PHMB at sites in the UK and the USA, no cases of dermatological problems have been reported to the occupational health unit at either site (reference: ECHA, Ref 33, 2011b). This information is in line with medical surveillance information during 2004 until 2007. 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 addition, employees, who work with skin sensitizers, participated in the “Skin Sensitizer Medical Surveillance Program”. These employees were examined every six months for signs of skin sensitization. During the observation period, there was no reported case of skin sensitization to PHMB (Ref 44, 2007).

6.10.3 Clinical patch tests and case reports

There are clinical patch tests available, which were performed in a varying numbers of patients showing signs of allergic contact dermatitis.

The working group of Schnuch et al. conducted patch tests on a total of 1554 male and female patients suspected to have contact allergies to medications and cosmetics in 1998 accordance to recommendations of the International Contact Dermatitis Research Group (ICDRG) and the German Contact Dermatitis Research Group (DKG). The patients were exposed to PHMB at 2.5% in an aqueous solution. 389 patients were exposed for 1 day and 1165 for 2 days. The skin reactions were scored on day 3. Six patients (0.4%) showed a skin positive reaction (+). One of

the reactions in a patient with atopic dermatitis may have been false-positive. The authors concluded that PHMB sensitization can be considered to be extremely rare (Ref 102, 2000). The same working group performed a follow up study. PHMB received as Cosmocil CQ was prepared for patch testing at concentrations of 2.5% and 5% PHMB in water. The preparations were tested in parallel in 1975 unselected patients from 1 July to 31 December 2005. Frequencies of sensitization (as % of patients tested) were calculated as crude proportions and additionally standardized for sex and age. Ten patients (0.5%) showed a positive skin reaction to PHMB at 2.5% and 16 patients (0.8%) to PHMB at 5%. However, the authors assumed that probably at least 4 reactions at 2.5% may be doubtful or irritant, i.e. false positive, as they were not confirmed by simultaneous reactions to higher concentrations. Potential causal exposures were assessed by a case by case analysis and by referring to surrogate markers of exposure in terms of concomitant reactions. Occupational exposures were identified as a probable cause of sensitization. Further risk factors included leg dermatitis and old age. In agreement with the previous study, the frequency of sensitization remained very low. The authors concluded finally, that it is very unlikely that exposure to cosmetics or personal care products may have played a role in the few cases sensitized (Ref 103, 2007). In agreement to the above studies, in an earlier study with very limited information, 2 out of 374 patients (0.5%) reacted positively to PHMB patch tested at 2.5% PHMB in water (ECHA, Ref 33, 2011b).

6.10.4 Photo-sensitization

Study Design:

Reference:	Ref 52, 1976
Date of report:	1976
Guideline/Method:	Adaptation of the repeat insult patch test (RIPT) procedure of Draize
Species:	Human
Group size:	30 volunteers (males, females, age range: <21 - >60 years)/26 completed (13 males, 13 females)
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	ADGM 3429 (Baquacil SB, 20% aqueous PHMB solution)
Vehicle:	Distilled water
Route:	Occlusive epicutaneous application on the upper arm
Induction:	Application of the test material solution at noon on three times per week (e.g., Monday, Wednesday, Friday) for 3 or 4 weeks for a total of 9 – 12 applications
Challenge:	6 weeks after initial application
Exposure duration:	24 h
Concentrations:	1% PHMB
Light source:	Direct rays of mid-day sun
Light exposure:	immediately after patch removal, 1 h
Skin readings:	during induction: prior to 2nd through 9th application and on Monday following 9th application48, 96 h after application After challenge: 48, 96 h after application
GLP:	Yes
Published:	No

Material and methods:

The potential of PHMB to cause light induced dermal toxicity was assessed in a human volunteer study using an adaptation of the repeat insult patch test (RIPT) procedure of Draize. A group of 26 volunteers were exposed to a 20% aqueous solution of PHMB. This solution was diluted further in water (1% v/v PHMB) and was applied three times per week for 3 or 4 consecutive weeks (giving a total number of applications of 9 or 12). Exposure was by means of a skin patch moistened with 0.4 mL of test solution applied to the upper arm. To increase the skin penetrating properties of the sample, sodium lauryl sulphate was added to the solutions to provide a final concentration of 0.01% in the patch solutions. The patches were applied at noon and removed 24 hours after application. Immediately after patch removal the test site was exposed to natural sunlight for one hour. Additionally, a challenge application was made 6 weeks after the initial exposure and the skin assessed 48 and 96 hours following application.

Results

During induction there was no evidence of skin irritation except for 1 individual, who showed a definite erythema following the 4th and 5th applications and minimal erythema following the 3rd and the 6th through the 12th application. No skin reaction was observed following challenge.

Conclusion

There was no indication for a photoallergic skin reaction and thus, PHMB did not elicit photo-sensitisation at a topical dose of 1% when tested in male and female human volunteers under the conditions of the study.

6.10.5 Overall conclusion on human data

In a human insult patch test, aqueous PHMB solutions up to a concentration of 5% PHMB did not show any undesirable primary skin irritation.

PHMB is capable of causing skin sensitisation in humans after repeated occlusive exposure at induction concentrations of at least 2% PHMB. Nevertheless, in over 30 years of manufacturing PHMB at sites in the UK and the USA, no cases of dermatological problems have been reported to the occupational health unit at either site. In addition, medical surveillance information during 2004 until 2007 of manufacturing and laboratory employees examined every six months for signs of skin sensitization, showed no reported case of skin sensitization to PHMB. Results from clinical patch tests (2.5 and 5% PHMB in water) indicated that skin sensitization from PHMB can be considered to be extremely rare. In addition, there was no indication of photo-sensitisation in an adapted repeat insult patch test (RIPT) at a topical dose of 1% PHMB in water in male and female volunteers.

6.11 Special investigations

Mechanistic *in vitro* study on liver haemangiosarcoma induction

Study Design:

Reference:	Ref 73, 2008
Date of report:	2008
Guideline/Method:	Explorative screening study
Test system:	SVEC4-1 0 mouse endothelial and RAW 264.7 mouse macrophage lines
Replicates:	2 - 3
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	No data

Concentrations:	Cytotoxicity assay in SVCC-10 cells: 0, 1, 2, 3, 4, 5 ppm Cytotoxicity assay in RAW macrophages: 0, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5 ppm Endothelial cell proliferation: 0, 0.5, 1, 2, 3 ppm Co-cultivation assays: 0, 0.75, 1.0 ppm Reactive oxygen species (ROS) assay: 0, 0.5, 0.75, 1 ppm
Vehicle:	Dulbecco's Modified Eagle Medium (DMEM)
GLP:	No
Published:	No

Material and methods:

PHMB was examined *in vitro* on how the interaction between macrophages and liver endothelial cells can induce endothelial cell proliferation. RAW 264.7 mouse macrophages were co-cultured with SVEC-10 mouse liver endothelial cells in various experimental conditions. The PHMB concentrations investigated ranged between 0.5 – 5 ppm. Prior to the main assays, the cytotoxicity of PHMB towards SVEC-10 and RAW 264.7 cells was examined by means of MTT (3-(4,5-dimethylthiazol-2-lyl)-2,5diphenyl tetrazolium). Thereafter, pre-activation of macrophages with PHMB or lipopolysaccharide (LPS) and/or co-culture in the presence of PHMB were studied. Endothelial cell proliferation was analysed by the incorporation of BrdU. Production of reactive oxygen species in macrophages treated with PHMB was investigated by measurement fluorescence intensity after addition of dihydrorhodamine and by evaluation of TNF-gamma and IL-6 in cell culture medium as quantified by ELISA.

Results and conclusion:

Under the condition of this *in vitro* study, PHMB had no direct effect on liver endothelial cell proliferation. PHMB did not activate macrophages and did not potentiate cell proliferation induced by LPS-activated macrophages.

Mechanistic *in vivo* study on liver haemangiosarcoma induction in mice

Study Design:

Reference:	Ref 73, 2008
Date of report:	2008
Guideline/method:	Explorative screening study
Species/strain:	Mouse/C57Bl
Group size:	5 male animals per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	No data
Dose levels:	0, 100, 200, 400, 1200, 4000 ppm
Route:	Oral (diet)
Exposure period:	7, 14 or 28 days
Exposure frequency:	daily
GLP:	No
Published:	No

Material and methods:

As chronic exposure of PHMB in mice caused increases in liver haemangiosarcoma accompanied by an increase in Kupffer cell pigmentation and Kupffer cell hyperplasia, this mechanistic study in mice was performed. Male C57Bl mice (5/group) were received diets

containing 0, 100, 200, 400, 1200 and 4000 ppm PHMB for 7, 14 or 28 days. Homogeneity of the diet was confirmed. Seven days prior to sacrifice, osmotic minipumps containing BrdU (20 mg/mL, 0.9% sterile saline) were implanted subcutaneously in mice for the determination of DNA synthesis. Following treatment, mice were sacrificed by CO_2 asphyxiation. Blood was collected by cardiac puncture in heparin-containing tubes. Plasma samples were obtained by centrifugation. Livers were perfused with PBS, removed, and weighted. A portion of liver from each mouse was fixed in 10% neutral-buffered formalin, embedded in paraffin and sectioned for immunohistochemical analysis. The remaining liver tissue was snap frozen in liquid nitrogen and stored at -80°C for oxidative stress analysis.

Results, discussion and conclusion:

The administration of PHMB at dietary concentrations of 0, 100, 200, 400, 1200 and 4000 ppm to male mice for 7, 14, or 28 days did not induce hepatotoxicity at any concentration or time point. At 4000 ppm a transient decrease in body weight and induction of thinning of the stomach wall was noted. At 28 days of exposure, no effect on body weight or liver weight was observed at any dose level. PHMB increased cell proliferation by means of increased DNA synthesis dose-dependently at 1200 and 4000 ppm. Cell proliferation was also increased at 1200 ppm following 14 days exposure. PHMB increased plasma endotoxin, a known activator of macrophages, at 1200 and 4000 ppm after exposure for 28 days and at 100 and 200 ppm after exposure for 14 days only.

Overall, the authors suggested that the effect of PHMB noted *in vivo* on liver endothelial cell growth at 1200 and 4000 ppm after dietary exposure of 28 days was induced by an indirect effect. The release of endotoxins at the same doses suggested that endotoxin mediated activation of macrophages may be involved. However, the causal relationship was not clearly demonstrated and the presence of endotoxins after intermediate exposure duration at lower doses questioned the relevance. Other mechanisms of action cannot be excluded. Under the conditions of this study, the increase in endothelial cell growth as measured by DNA synthesis occurred in a dose-related fashion but with a clear threshold at 400 ppm.

Sensory irritation study

Study Design:

Reference:	Ref 93, 1993
Date of report:	1993
Guideline/method:	Comparative sensory irritation study
Species/strain:	Mouse/Alpk:APfCD-1
Group size:	5 female animals per group
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	no data on batch (20% aqueous PHMB solution)
Concentrations:	a) 5, 50, 200 mg/m³ (analysed: 11.7, 62.9, 208 mg/cm³) b) 10 ppm (in use concentration, corresponding to 10000 mg/m³ particulate concentration)
Vehicle:	b) synthetic pool water
Route:	Inhalation (nose only)
GLP:	No
Published:	No

Material and methods:

PHMB (20% aqueous solution) and another compound were comparatively examined for their sensory irritation potential in mice after inhalation. Initially, test atmospheres were generated from PHMB as supplied and groups of 5 female Alpk:ApfCD-1 mice were exposed nose only to concentrations of 5, 50 and 200 mg/m³. PHMB was delivered via a peristaltic pump, to a glass jet atomiser and the resultant atmosphere fed directly into a two tier nose only chamber. Compound and air flow rates through the atomiser were altered as required in order maintaining target concentrations. During exposure, atmospheres were sampled and analysed by colorimetric titration. The respiration rate of the mice was measured for each target concentration using pressure plethysmography and the RD_{50} (concentration which causes 50% depression in respiratory rate) was calculated. In order to assess the sensory irritant potential of “in use” concentrations of PHMB, atmospheres were generated from PHMB and the comparative compound diluted in synthetic pool water to on a final formulation concentrations of 50 ppm (corresponding to 10 ppm PHMB). Groups of five female Alpk:APfCD-1 mice were exposed, nose only, to target particulate concentrations of 10000 mg/m3 (maximum output from the atmosphere generation equipment) formulation or to synthetic pool water alone.

Results:

The achieved concentrations of PHMB were stable and acceptable close to the target concentrations. Small differences seen between target and analysed concentrations reflected difficulties in generation of relatively low particulate concentrations of PHMB. The aerodynamic particle size distribution showed MMAD values of 2.52, 3.08, 4.32 µm with a geometric standard deviation of 2.84, 2.39 and 2.49 for the target concentrations of 5, 50 and 200 µg/m³, respectively. The mean respiratory rate depression was 12±4%, 20±7% and 40±15% for the target concentrations of 5, 50 and 200 µg/m³, respectively. The RD_{50} value was calculated as 264 µg/m³. A non-linear relationship of concentration to respiratory rate depression was observed in mice exposed to 208 mg/m³. In one animal, there was a marked depression in respiratory rate, from which the animal showed little recovery. There was no significant difference between the respiratory depression exposed to "in use" concentrations of PHMB and to those exposed to synthetic water alone. This phase was conducted at the maximum output of the aerosol generation equipment, which achieved a very high particulate concentration. Even under these extremely artificial conditions, the only effects seen were consistent with exposure to very high inert aerosol concentrations and no influence of PHMB was apparent. Therefore, PHMB diluted in synthetic water at "in use" concentrations, would be not expected to cause sensory irritation.

Conclusion:

Under the condition of this study it was shown that PHMB was sensory irritant of less potency in mice. However, the results of the “in use” concentration showed that PHMB diluted in synthetic water is not expected to cause sensory irritation.

Mammalian cell transformation assay:

Study Design:

Reference:	Ref 111, 1980
Date of reports:	1980
Guideline:	Mammalian cell transformation assay according to Styles, 1977
Test system:	Baby hamster kidney fibroblasts (BHK21/C13)
Replicates:	quadruplicate plates, two independent experiments
Test substance:	Polyaminopropyl biguanide (PHMB)
Batch:	Bx2125 (Vantocil IB, 19.6% aqueous PHMB solution)

Concentrations:	Experiment I: +S9 mix: 0, 0.25, 2.5, 25, 250, 2500 µg/mL Experiment II: +S9 mix: 0, 25, 250, 750, 1500, 3000 µg/mL
Vehicle:	DMSO
Positive Controls:	Experiment I: Benzidine: 0.25, 2.5, 25, 250, 2500 µg/mL Experiment II: Acrylonitrile: 0, 0.23, 2.3, 23, 230, 2300 µg/mL
Negative controls:	Vehicle plus S9 mix, cell suspensions ±S9 mix
GLP:	Yes
Published:	No

Material and methods:

PHMB (batch: Bx2125, 19.6% aqueous PHMB solution) was tested for its potential to induce cell transformations mutagenicity in baby hamster kidney fibroblasts (BHK21/C13). PHMB was dissolved in DMSO, which was also used as vehicle control and was investigated over a dose range of 0.25 – 2500 µg/mL in experiment I and 25 – 3000 µg/mL in experiment II in quadruplicate cultures. All experiments were conducted in the presence of metabolic activation (S9 mix of Aroclor 1254-induced rat liver). For control purposes, the vehicle (DMSO) plus S9 mix and cell suspensions ±S9 mix was examined as negative controls and Benzidine and Acrylonitrile were used as positive controls in experiment I and II, respectively. Cell suspensions were prepared and distributed to centrifuge tubes and the metabolic activation system and test solutions were added and incubated at room temperature for 3 hours. Thereafter, the tubes were centrifuged and the cell pellets were resuspended in Dulbecco's modification of Eagle's medium. Aliquots of the cell suspension were transferred to petri dishes and examined for survival or transformation of the cells after an incubation period of either 6 – 9 or 14 – 21 days in a humidified CO_2 incubator at 37 °C, respectively. Thereafter, the cell colonies were stained and counted using an automatic electronic colony counter (Artek®).

Results:

Cytotoxicity was observed in the survival and transformation assay at concentrations ≥250 µg/mL in experiments I and II. The number of transformed cell colonies did not differ between plates containing the test substance and those containing the negative controls. The positive controls induced large numbers of transformed colonies demonstrating the sensitivity and suitability of the test system.

Conclusion:

PHMB did not induce cell transformations in Baby hamster kidney fibroblasts (BHK21/C13) in the presence of S9-mix when tested up to cytotoxic concentrations.

6.11.1 Overall conclusion special investigations

In mechanistic *in vitro* studies it was shown that PHMB showed neither a direct nor indirect effect on hepatic cell proliferation. Mechanistic *in vivo* studies in mice suggested an indirect effect for endothelial cell growth in the liver but a clear causal relationship could not be demonstrated. In any case, a clear threshold was obtained.

PHMB did not induce cell transformations in a standard mammalian cell transformation assay.

7 Risk assessment

Content redacted as EU specific

7.1 Overall Conclusion

Content redacted as EU specific

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9. Data base search for references

A comprehensive data base research on the toxicological profile of PHMB was carried out using SCIFINDER, which searched CAPlus and MEDLINE for relevant references.

All hits relevant for risk assessment of PHMB were included in the present safety evaluation. The complete print outs covering all hits is attached.

PHMB data base search until April 2013, unpublished data, CBI

03/02/2017

Monographs Proof Report

- P -

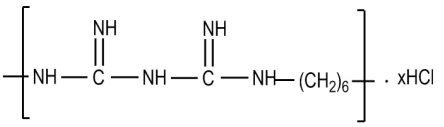
POLYAMINOPROPYL BIGUANIDE

INCI Monograph ID: 3731

CAS No.: 32289-58-0

Empirical Formula:
$(C_8H_{17}N_5)_n \cdot xClH$

Definition: Polyaminopropyl Biguanide is the organic compound that conforms to the formula:



See Sections 20 to 22 for the Japanese, Chinese, and Korean translations of this INCI Name.

Information Sources: CLP 616-207-00-X, EC(V-28), INN, MHLW-331/3, MI, UNII: 322U039GMF

Chemical Class: Synthetic Polymers

Reported Function: Preservative

Ingredient Source: Synthetic

Technical/Other Names:
polihexanide (INN)
Poly(hexamethylenebiguanide) hydrochloride
Poly[iminocarbonimidoyliminocarbonimidoylimino-1,6-hexanediyl], hydrochloride

Trade Names:
Cosmocil CQ (Lonza Personal Care)
Microcare MBG (Thor Specialties, Inc.)

Trade Name Mixtures:
Euxyl K 702 (Schulke & Mayr)
Microcare MTB (Thor Specialties, Inc.)

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: February 21, 2017

SUBJECT: Comments on the Scientific Literature Review: Safety Assessment of Polyaminopropyl Biguanide as Used in Cosmetics (release date: February 14, 2017)

Key Issue

The summary of the new dermal penetration study in the 2016 preliminary SCCS opinion does not accurately reflect the study. The description of this study begins on p.35 of the SCCS opinion. In this study, two test preparations were used, aqueous micellar solution and oil/water emulsion. In one part of the study, the skin was exposed for 24 hours and penetration determined directly after exposure. In the second part of the study, the skin was exposed for 24 hours and then there was an additional 72 hour period to determine if the material in the skin moved to the receptor fluid. The results indicated that most of the radioactivity in the skin stayed in the skin during the additional 72 hour period. The SCCS said (p.39): "Therefore the SCCS agrees that the amount found in the epidermis may be excluded from the total absorbed dose." Although this is suggested in CIR report Table 11, it is not made clear in the text (Dermal Penetration section) or in Table 4.

Additional Considerations

Cosmetic Use - Please state the specific FDA product categories (hair grooming products and skin cleansers) in which Polyaminopropyl Biguanide is being used at the maximum reported concentrations.

In the following sentence "in cosmetic products" needs to be deleted as use in cosmetics does not impact whether or not a substance is classified as CMR. "CMR substances are substances that are classified as carcinogenic, mutagenic, or toxic for reproduction in cosmetic products."

Dermal Penetration - As stated above, the Dermal Penetration section needs to revised to accurately describe the most recent *in vitro* human dermal penetration study summarized in the 2016 preliminary SCCS opinion. An additional 72 hours did not significantly

increase the amount of material in the receptor fluid. Therefore, the amount found in the skin can be considered as not absorbed.

Risk Assessment - What was the value for the acute population adjusted dose (aPAD) used by the EPA?

Acute, Inhalation - Please state the duration of exposures in the text.

Short-term, Inhalation - Please state the hours/day, days/week the rats were exposed.

Chronic, Dermal, Summary, Table 11 - What was the mortality rate for the other dose groups? Did they really complete microscopic examinations of just the eye and thyroids? It should be stated if other organs were also examined.

Chronic Oral, Summary, Table 11 - The MOS calculations should be presented in the Risk Assessment section. It is not clear what the MOS values represent, e.g.,46 is the value calculated assuming all cosmetics contain 0.3% Polyaminopropyl Biguanide, and the higher values were calculated assuming all cosmetics contain 0.1% Polyaminopropyl Biguanide. The assumptions for dermal penetration used in these MOS calculations should also be stated.

Risk Assessment - What NOAEL did EPA use to calculate the MOEs?

Developmental and Reproductive Toxicity Studies - Please state the media, e.g., food or water, in which the rat NOAECs of 1000 and 1300 ppm were identified. What was the duration of the inhalation study reporting degeneration of the seminiferous tubules?

Carcinogenicity, Dermal - What was the mortality in the other treatment groups?

Sensitization, Summary, Table 15 - In the text, please include the reference for the local lymph node assay (LLNA). Table 15 does not provide any details of the LLNA and states that Polyaminopropyl Biguanide was a weak sensitizer, while the Sensitization section and the summary state the results of the LLNA as "non-sensitizer".

Ocular Irritation - Please state the volume (100 µl) and duration of exposure (1 minute) used in the *in vitro* study of human and rabbit eyes.

Retrospective Multicenter Studies - As the original SCCS opinion (reference 4) is a secondary source, it is not clear why it is presented as a reference for patient multicenter studies.

Summary - The Summary should give some indication on how much Polyaminopropyl Biguanide penetrates the skin (about 0.02% in 24 hours).

Please state the route of exposure for the 60-day study in rats.

The HRIPTs should also be mentioned in the Summary.

Table 2 - It is not clear why this table states "liquid" under heading physical form and "solid" under the heading color. The most useful molecular weight distribution information from the recent preliminary SCCS opinion should be added to this table. The preliminary opinion states 6% is <500; 14.1% is between 500 and 1000; and 75.8% is greater than 1000 dalton.

Table 4 - As the composition of the receptor fluid impacts how much penetrates the skin, the identity of the receptor fluid should be stated for all *in vitro* dermal penetration studies.

As stated above, the summary of the new dermal penetration study needs to be significantly revised. For example, in the Ingredient column, it incorrectly states that the

exposure concentration was "0.1% w/w in oil-in-water emulsion" for the study with the extended 72 hour collection period. For this study, the concentration was 0.3% in both an aqueous micellar solution and an oil/water emulsion. It is not clear what is meant by "absorbed dose". Is this the amount found in the receptor fluid? Two values are given for mass balance and it states "respectively", but it is not clear what these values represent.

Table 5 - In the Results column for the last study, please correct: "At a of 200 g..". It is not clear that g is the correct units as the Protocol column says 200 $\mu l/cm^2$. Please state who concluded that it "was not possible to derive a realistic dermal absorption rate from this study." The last two studies cited to the original SCCS opinion (reference 4) are cited to the same reference in the opinion (opinion reference 10). The reference title "Characterisation of the Urinary Polymer-related Material from Rats given Poly[biguanide-1,5-diylhexamethylene hydrochloride" suggests that all the studies reported in this paper were completed in rats (Table 5 says "Animal species not stated"). The following link is found when Google is used to search for this reference title: http://onlinelibrary.wiley.com/doi/10.1002/macp.1976.021770902/full. As this is a published study, it would be helpful if the studies were cited to the primary reference rather than the SCCS opinion.

In the second last study of Table 5, it says that 3 male rats were used. The results column gives values for 3 weeks, 5 weeks, and after a 3 week recovery group. Did they look at just one rat at each time point, or were there 3 rats/group?

In the last study, when after dosing was the whole body autoradiography completed?

Table 6, Table 9, Table 11, Table 12, Table 14, Table 15 - In a number of tables the term "SPF strain" is used. Specific-pathogen-free is not a "strain". It is "a term used for laboratory animals that are guaranteed free of particular pathogens."

Table 7 - Please provide the mg/kg dose or the dose volume used in the study of 25% aqueous Polyhexamethylene Biguanide Hydrochloride in 3 female rats (reference 17).

Table 9, oral, last study - What were the "few deleterious effects in internal organs"?

Table 11 - Please look at the reference section of reference 19 (a review) to see if the last two studies in Table 11 are reported elsewhere in this table.

Table 12 - The two studies in Alderley Park mice appear to be the same study (one is cited to the original SCCS opinion and one is cited to the new SCCS opinion). The results in the description of the first study Alderley Park mouse study appear to be the results for the rat (Alderley Park) rather than the mouse study.

Table 14, Dermal Studies - Please check the reference sections of the SCCS opinions. Were there really two 80 week dermal studies in Alderley Park mice at doses of 0, 0.6, 6 and 30 mg/mouse? The first presentation of this study does not include a reference.

Regarding the oral study in female Swiss mice, please state who concluded: "Data considered to be of low reliability due to high mortality."

Table 15, Sensitization - As a local lymph node assay (LLNA) is completed in mice, it is not clear why it needs to be placed under a In Vivo Assay subheading. If available, please

provide more details about the LLNA. It is cited to reference 25 which seems to be a secondary reference. If the primary reference was published, it should be retrieved. It should be stated if the primary reference was an unpublished study cited in this secondary reference.

Please correct: "fait erythema"

Is the abstract (reference 26) really the correct reference for two multicenter studies (one in the United Kingdom, one in Germany)?