



Scientific Committee on Consumer Safety

SCCS

OPINION ON

the safety of poly(hexamethylene) biguanide hydrochloride (PHMB)

The SCCS adopted this opinion at its 6th plenary meeting
on 18 June 2014

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat. They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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This opinion has been subject to a commenting period of minimum four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

Keywords: SCCS, scientific opinion, poly(hexamethylene) biguanide hydrochloride, polyaminopropyl biguanide (PHMB), Regulation 1223/2009, CAS No. 32289-58-0 or 27083-27-8).

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1. BACKGROUND

Poly(hexamethylene) biguanide hydrochloride (PHMB) (CAS Nos. 32289-58-0 or 27083-27-8, INCI Polyaminopropyl Biguanide), is authorized as preservative in cosmetic products at a maximum permitted concentration of 0.3%, according to Annex V of the Regulation (EC) No. 1223/2009¹.

Polyaminopropyl Biguanide (PHMB) is classified as CMR2 according to the Commission Regulation (EU) No. 944/2013² of 2 October 2013 amending for the purposes of its adaptation to technical and scientific progress the Regulation (EC) No. 1272/2008³. In particular, in recital 7 of Commission Regulation (EU) No. 944/2013 it is reported that for PHMB the specific hazard 'acute toxicity (inhalation)' is under evaluation by the RAC. The classification will apply from 1st January 2015. As a consequence, PHMB would be prohibited from 1st January 2015 as cosmetic ingredient according to art. 15 (1) of Regulation (EC) No. 1223/2009 on cosmetic products.

However, article 15 (1) of Regulation (EC) No. 1223/2009 states that '*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*'.

In January 2014, the Commission received a submission from Cosmetics Europe⁴ on PHMB, intended to demonstrate the safety of the ingredient when used as a preservative in cosmetics products up to a maximum concentration of 0.3%.

After the commenting period which followed the publication of SCCS/1535/14 on 18 June 2014, a report on an *in vitro* mammalian cell gene mutation test and a draft of a new *in vitro* percutaneous absorption study were provided by the applicant along with submission dossier II and further references. These documents were used for revising the opinion.

2. TERMS OF REFERENCE

1. *In view of the above, and taking into account the scientific data provided, SCCS is requested to give its opinion on the safety of Polyaminopropyl Biguanide (PHMB) when used as a preservative in cosmetics products up to a maximum concentration of 0.3%.*
2. *SCCS is requested to address any further scientific concerns on the ingredient Polyaminopropyl Biguanide (PHMB) in particular regarding its use in spray formulation.*

¹ <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF>

² <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:261:0005:0022:EN:PDF>

³ <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:353:0001:1355:en:PDF>

⁴ Cosmetics Europe - European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Chemical name: Polyhexamethylene biguanide hydrochloride (PHMB)*

INCI Name: Polyaminopropyl Biguanide

*Abbreviation PHMB has been used throughout this opinion

3.1.1.2 Chemical names

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

Other chemical names

Poly(hexamethylenebiguanide hydrochloride)

Poly(iminocarbonimidoyliminocarbonimidoylimino-1,6-hexanediyI), hydrochloride

Poly(iminoimidocarbonyl-iminoimidocarbonyl-iminohexamethylene), hydrochloride

Poly(iminoimidocarbonyliminoimidocarbonyliminohexamethylene) hydrochloride

3.1.1.3 Trade names and abbreviations

Baquacil

Caswell No. 676

Cosmocil CQ

EPA Pesticide Chemical Code 111801

PHMB

Polihexanide

Polihexanido

Polihexanidum

Polyhexanide

PP 073

UNII-322U039GMF

Vantocil IB

Vantocil TG

Reference: Submission file, therein cited: TOXNET 2013

3.1.1.4 CAS / EC number

CAS: 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-hexanediyIbis(N'-cyanoguanidine) and 1,6-hexanediamine).

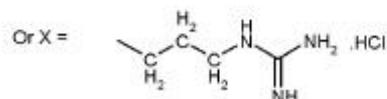
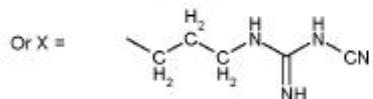
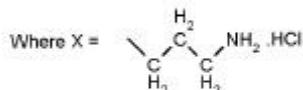
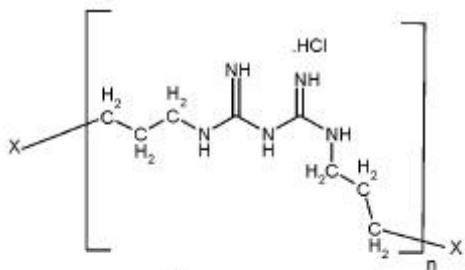
CAS-No 32289-58-0 expresses the PHMB as the resultant polymer.

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

2nd Revision of the opinion on the safety of poly(hexamethylene) biguanide hydrochloride or polyaminopropyl biguanide (PHMB) in cosmetic products

Ref.: 2; 30

3.1.1.5 Structural formula



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

Ref.: 2

3.1.1.6 Empirical formula

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

Ref.: 2

3.1.2 Physical form

Off-white to pale yellow powder with strong ammonia smell at 20° C and 101.3 kPa

Ref.: 2; 30

Very pale yellow to pale yellow, lumpy solid; no obvious odor.

Ref.: 5

Pale-yellow glass-like solid (technical grade PHMB).

Ref.: 22

3.1.3 Molecular weight

Molecular weight: > 700 g/mol (submission)

2670 – 2960 (weight average molecular weight) Da (Ref. 5)

3686 – 4216 (weight average molecular weight) Da (Ref.22)

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 g/mol.

Ref.: 35

3.1.4 Purity, composition and substance codes

> 94.2 % (w/w in dry weight)

Ref.: 5; 22; 30; 35; 36

3.1.5 Impurities / accompanying contaminants

Table 1: Impurities reported for PHMB

Impurity [% (w/w)]	PHBM, DS6261 (Ref. 5) Samples: ASG 10320307, ASG 10320308, ASG 10320309, ASG 10320310 ASG 10320311	Vantocil P, (Ref. 35) Samples: DR4529; DR4532; DR4535; DR4538; DR4541	Vantocil P, (Ref. 36) Samples: DR4529	PHMB, DS 6274 (Ref.: 23-26)	Five different batches of technical grade PHMB (Solid PHMB) (Ref. 22)
HMD	0.26 – 0.32	0.09 – 0.12	0.1	< 0.1	< 0.1
HMDA	0.6 – 1.2	0.13 – 0.16	0.16	0.5	0.07 – 0.29
Impurity A	1.67 – 3.26	2.3 – 2.8	2.8	1.2	0.07- 0.29
Impurity B + C	0.9 – 1.08 (C only)	1.1 – 1.3	1.3	0.6	0.5 – 1.4

HMD: polymerization starting material Hexamethylenediamine [H₂N(CH₂)₆-NH₂]

HMDA: polymerization starting material Hexamethylenebisdicyandiamide

Impurity A: possibly N-(6-aminohexyl)-N'-(6-guanidinoethyl)guanidine (Ref. 35); according to Ref. 36, impurity A can be accounted in part by HMDA but ca 0.2 % is due to other components present (proposed impurity C, N-cyano N'-(6-N-cyanoaminohexyl)guanidine and N-Cyano N'-(6-aminoethyl)guanidine).

Impurity B: possibly 1,6-diguanidinothexane dihydrochloride (Ref. 35)

Impurity C: possibly N-cyano-N'-(6-guanidinoethyl)guanidine hydrochloride (Ref. 35)

Nine other impurities in concentrations 0.01 -0.9 % (w/w), total 1.2 -2.7% (w/w) were found in 5 technical grade PHMB by HPLC/MS analysis. On the basis of mass spectra, these impurities were considered to be "active substance related" (Ref. 22).

Water content in 5 batches of technical grade PHMB: 3 – 5 % (Ref. 22).

Trace metal contents (in ppm, w/w) in five different batches of PHBM: Cd: < 0.25; Cr: < 0.25 – 0.7; Co: < 0.25; Fe: 14 – 40; Pb < 2; Zn: 370 – 540; As <2 and Hg < 0.2.

Ref. 5, 22

3.1.6 Solubility

Water solubility:

41 ± 1 % [w/w] at 25° C (Ref. 2)
39.0 – 43.4 % at 23.4° C (Ref. 5)
426 g/l (Toner, 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

Ref.:30

SCCS Comment

The method used for the determination of water solubility was not reported.

3.1.7 Partition coefficient (Log P_{ow})

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

3.1.8 Additional physical and chemical specifications

Melting point:	a) 78.9- 136.3° C (Ref.: 2) b) decomposes without melting at 205 – 210 °C (Ref. 30)
Boiling point:	decomposes at 205-210°C before boiling (Ref.: 30)
Flash point:	
Vapour pressure:	a) 1.32×10^{-7} Pa (20°C) and 4.11×10^{-7} Pa (25°C) (estimated according to OECD TG 104) (Ref. 16) b) 6.0×10^{-8} Pa (20°C) and 2.0×10^{-7} Pa (25°C) (estimated according to OECD TG 104) (Ref. 69)
Relative Density:	1.20 ± 0.0025 (at 20 ± 0.5°C) (Ref.: 30)
Viscosity:	/
pKa:	4.19 at 25°C (Ref.: 30)
Refractive index:	/
pH:	4.36 at 21.7° C (Ref.:5)
UV_Vis spectrum:	wavelength maximum at 236 nm (Ref.:5)

SCCS comment

The SCCS notes that melting point as given in Ref. 2 (which gives reference to a non-available report [Bannon 2008]) spans a large range. This might be due to the heterogenous nature of the polymer. Differing information is available from Ref. 30 and 5 where it is mentioned that the substance decomposes without melting at 205 – 210 °C.

3.1.9 Homogeneity and Stability

In an attempt to accelerate the ageing of the solid substance by heating at 54 ± 2° C for 14 days, the substance has been shown to be stable and there was no significant degradation. An ambient shelf-life of at least 2 years was derived from this study result.

Ref.:5

The stability of PHMB in deionised water has been established for at least 6 weeks.

Ref. 72

In Ref. 27 it was stated that nominal concentrations (expressed as Vantocil, i.e. 20 % PHMB) of 0.1, 35 and 80 mg/ml would be stable for the duration of the test.

Ref.: 27

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.: 47

General SCCS comments on physico-chemical characterisation

It is not known whether the water solubility of PHMB was determined by EU Method A.6.

The SCCS notes that the melting point as given in Ref. 2 (which gives reference to a non-available report (Bannon 2008)) spans a large range. This might be due to the heterogenous nature of the polymer. Differing information is available from Ref. 30 and 5 where it is mentioned that the substance decomposes without melting at 205 – 210 °C.

Molecular weight distribution of the polymer was not provided.

3.2 Function and uses

PHMB is supported under Directive 98/8/EC for uses as a disinfectant.

Ref.:2

PHMB is used as a preservative and as an antimicrobial agent. As a preservative, PHMB is used in cosmetics, personal care products, fabric softeners, contact lens solutions, hand washes, and more.

In cosmetics, PHMB is used as a broad spectrum preservative. It is freely water soluble and therefore widely used in water-based products which are most susceptible to microorganism growth. It has an excellent activity against a wide range of Gram positive and Gram negative bacteria, fungi and yeasts and is particularly effective against microorganisms such as *Pseudomonas species*, which are difficult to control.

PHMB is also used to preserve wet wipes; to control odour in textiles; to prevent microbial contamination in wound irrigation and sterile dressings; to disinfect medical/dental utensil and trays, farm equipment, animal drinking water, and hard surfaces for food handling institutions and hospitals; and to deodorize vacuums and toilets. PHMB is used in antimicrobial hand washes and rubs and air filter treatments as an alternative to ozone. PHMB is also used as an active ingredient for recreational water treatment, as a chlorine-free polymeric sanitizer, which is effective against a wide variety of microorganisms.

Further reported uses of PHMB are purification of swimming pool water, beer glass sanitisation, solid surface disinfection in breweries and short-term preservation of hides and skins.

Ref.: submission dossier; 63, 28

3.3 Toxicological Evaluation

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Guideline:	OECD TG 425
Species/strain:	rat, Sprague-Dawley
Group size:	/
Test substance:	PHMB DS6274
Batch:	PC 100-02
Purity:	certificate of analysis was not attached; submission states 96.0 %
Vehicle:	distilled water
Dose levels:	550 and 2000 mg/kg bw
Dose volume:	20 ml/kg bw
Administration:	gavage
GLP:	yes
Study period:	2002

A total of 6 female animals were dosed individually in sequence with at least 48 hours between the animals at dose levels of 2000 or 550 mg/kg bw. All surviving animals were observed for 14 days post-dose.

All three animals treated with 2000 mg/kg were found dead during the day of dosing or one day after dosing. No deaths were noted at a dose level of 550 mg/kg.

Hunched posture and pilo-erection were noted in 2 animals treated with 2000 mg/kg just after dosing. Signs of systemic toxicity also noted 1 day after dosing in 1 animal treated with 2000 mg/kg were lethargy, ataxia, decreased respiratory rate, laboured respiration, ptosis and tiptoe gait.

There were no signs of systemic toxicity noted in animals treated with 550 mg/kg. The surviving animals showed expected gains in bodyweight over the study period. 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.

An acute oral LD₅₀ of 1049 mg/kg bw was derived from the study.

Ref.:23

Guideline:	Considered equivalent to OECD 401 but without gross necropsy
Species/strain:	Rat, Alderley Park
Group size:	5 / sex/ dose
Test substance:	Vantocil P (20 % aqueous solution of PHMB)
Batch:	BX 791/2[ADGM 1021/79]
Purity:	no information given
Vehicle:	deionised water
Dose levels:	700, 1000, 1500, 2000, 2500, 3000, 3500 and 5000 mg/kg bw
Administration:	stomach tube
GLP:	yes
Study period:	1979

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Animals were fasted for 16 – 20 hrs and then given various doses of an aqueous solution of PHMB by stomach tube at a standard volume of 10 ml/kg. Animals were observed for 14 days. No deaths occurred in animals dosed at 700, 1000 or 1500 mg/kg bw. Signs of toxicity could be observed in all dose groups and consisted of salivation, lacrimation, piloerection and in subdued appearance for some animals. At 1500 mg/kg bw, further signs of toxicity were wheezing and staining around the mouth. Toxic signs did not persist beyond day 7 or 8 of the study. Cumulative mortalities in male rats were 1 at 2000 mg/kg bw, 2 at 2500 mg/kg bw, and 4 at 3000, 3500 and 5000 mg/kg bw, respectively. In female rats, cumulative mortalities were 1 at 2000 mg/kg bw, 2 at 2500 mg/kg bw, and 5 at 3000 mg/kg bw, 4 at 3500 mg/kg bw and 5 at 5000 mg/kg bw. LD₅₀ values of 2747 mg/kg and 2504 mg/kg were derived for male and female rats, respectively corresponding to approximately 549 and 501 mg/kg bw a.i. in male and female animals, respectively.

Ref.: 55

3.3.1.2 Acute dermal toxicity

Guideline:	OECD TG 402 (EU B.3)
Species/strain:	Rat, Sprague-Dawley
Group size:	5 males and 5 females
Test substance:	PHMB DS6274
Batch:	PC 100-02
Purity:	certificate of analysis was not attached; submission states 96.0 %
Vehicle:	distilled water
Dose levels:	5000 mg/kg bw
Administration:	dermal, semi-occlusive
GLP:	yes
Study period:	2002

Clipped skin (10 % of the total body surface area of the dorsal and dorso-lateral parts of the trunk) of animals was treated with a single dose of 5000 mg/kg bw of PHMB (moistened with distilled water). The application area was covered by semi-occlusive dressing for 24 hours. Body weights were determined shortly before administration 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 0.5, 1, 2 and 4 hr after removal of the semi-occlusive dressing and subsequently once per week for 14 days. Animals were sacrificed after the observation period and gross pathological examination was performed.

No mortality occurred, no signs of systemic toxicity were observed and body weight gain was not affected. Very slight to well-defined erythema was noted at the treatment sites of all animals one and two days after dosing. Very slight erythema persisted at the treatment sites of two animals three days after dosing. Hemorrhage of dermal capillaries was noted at the treatment sites of eight animals one and two days after dosing. Small superficial scattered scabs were noted at the treatment sites of all animals. No abnormalities were noted at necropsy.

An acute dermal toxicity > 5000 mg/kg bw was derived from this study.

Ref.: 24

Guideline:	precedes OECD guideline but considered mainly in accordance with OECD TG 402
Species/strain:	Rabbit, White New Zealand
Group size:	2 males and females
Test substance:	Vantocil P (20 % aqueous solution of PHMB)
Batch:	BX 791/2[ADGM 1021/79]
Purity:	no information
Vehicle:	/
Dose levels:	2 ml of a 20 % aqueous solution

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Administration: dermal, occlusive
 GLP: yes
 Study period: 1979

Test substance was applied to the shorn area (ca. 150 x 130 mm) of the dorso-lumbar region and held in place by an occlusive dressing. At the end of the 24 hr contact period the dressings were removed, contact sites were cleaned with water and animals were observed for 14 days. No deaths occurred and it was concluded that the dermal LD₅₀ of the 20% solution is > 2 ml/kg bw, i.e. higher than 400 mg/kg bw a.i.

Ref.: 55

3.3.1.3 Acute inhalation toxicity

Two acute inhalation toxicity studies have been performed with PHMB. The studies are not available for evaluation by the SCCS. The studies have been evaluated by ANSES and RAC and have been described in a French proposal for classification and labelling of PHBM (Ref.: 2) as well as in the RAC background document to the opinion proposing harmonised classification and labelling of PHBM (Ref. 30) and presented here as citations from Ref. 2.

Study 1:

"In an acute inhalation study on a formulation containing 20.6 (% w/w) PHMB (but with no information on the non-active ingredients), Alpk:APfSC rats (five/sex) were exposed by nose-only for 4 hours to a single dose of 1.76 mg/l of the formulation, which corresponds to 0.36 mg/l of PHMB (mass medium aerodynamic diameters were 1.8-2.0 µm with a geometric standard deviation of 2 µm). (Arch Chemicals, subsequently submitted some unclear information on the non-active ingredients: 14% EDTA, 27% propylene (?) and water). Three hours after the exposure one male died (out of ten animals in total). All females and most males demonstrated respiratory stress 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. It is not possible to establish an LC50 for the formulation or for PHMB based on this study, but it could be estimated to be higher than 0.36 mg/l for PHMB."

Study 2:

"Wistar CRL:(WI) rats (n=5/sex/concentration in the main study) were exposed nose-only to an aerosol of PHMB (purity 99.6%) in aqueous solution in a GLP, OECD 403-compliant acute inhalation toxicity study (confidential reference, 2012). 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 the 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, ronchus, partial ptosis, decreased activity, increased respiratory rate, sneezing). Transient body weight decrease was observed but no test-item related macroscopic findings. Exposure levels in the main study were 0.1, 0.3 and 0.5 mg/l PHMB for 4 hours.

Mortality is summarised in table 2 below.

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Table 2: mortality of Wistar rats after inhalation of 0.1, 0.3 and 0.5 mg/l PHMB for 4 hr.

Concentration in PHMB	Mortality in males	Mortality in females	Comments
0.1 mg/L	0/5	0/5	-
0.3 mg/L	3/5	0/5	One male died at the end of the exposure and two males were found dead on the day following the exposure on Day 1.
0.5 mg/L	5/5	3/5	Male animals died immediately following the end of exposure (two males) or 1 hour following the end of exposure (two males) while one male and three female rats were found dead approximately 7 hours following the end of exposure.

Clinical signs:

At a concentration of 0.1 mg/L, the main clinical signs were observed on Day 0 and included: slight to moderately laboured respiration (all males and 3 of 5 females), rhonchus (2 of 5 males and 3 of 5 female), decreased activity (all males), hunched back (4 of 5 females) and increased respiratory rate (all females). Following the exposure on Day 1, the respiratory signs ceased and slightly laboured respiration and/or rhonchus (noisy respiration) was noted in two males only, with sneezing observed in all males for 3-4 days (up to Day 5). In all males and in 2 of 5 females weak body condition was noted during Days 1 and 2.

In all animals exposed to a concentration of 0.3 mg/L, slight to moderately laboured respiration was noted during the exposure. Following the exposure on Day 0, severely laboured respiration with rhonchus was noted in 4 of 4 males. In all females, a moderately laboured respiration was accompanied by slightly increased respiratory rate and noisy respiration was noted for 3 of 5 females. Slight to severe decreased activity was observed in 4 of 4 males and in 4 of 5 females. In addition, in a single animal, partially closed eyelids and moderate ataxia were observed. One male which displayed severe signs like severe decreased activity and prostration on Day 0, had severe laboured respiration with gasping and rhonchus, decreased activity and ruffled coat before death in afternoon on Day 1. On the following days the clinical signs ceased in surviving males and only slightly laboured respiration, slightly increased respiratory rate and weak condition were observed up to Day 3. In females, weak body condition was noted in 4 of 5 females on Days 1 and 2. In addition, in one female ruffled coat was observed on Days 2-3 and sneezing on Day 3.

At 0.5 mg/L the main clinical signs included: moderately to severely laboured respiration with noisy respiration up to gasping, increased respiratory rate and decreased activity. The clinical signs were observed following the exposure on Day 0. In two females, laboured respiration was observed up to Day 7. The respiration was moderately/severely laboured for 2-3 days after the exposure and in one animal accompanied by gasping and noisy respiration up to Day 6. In addition, hunched back and weak condition were noted up to Day 6. Sneezing was observed up to necropsy on Day 14.

Effect on body weight:

At 0.1 mg/L, approximately 8-11% body weight loss was observed in all males following the exposure on Day 1. The body weight returned to the initial values mostly on Day 7, in 2 of 5 animals between Days 7-14. In females slight body weight loss (about 4-6%) was recorded for 3 of 5 animals on Day 1. The body weight returned to the initial values approximately on Day 3 (in one female on Day 7).

At 0.3 mg/L, body weight loss was observed in surviving males (approximately 8-13%) and in all females (approximately 4-9%) following the exposure on Day 1. The body weight returned to the initial values between Days 3 and 7. At 0.5 mg/L, approximately 10-12% body weight loss was observed in surviving females following the exposure on Day 1 and 3. The body weight returned to the initial values between Days 7 and 14.

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Necropsy:

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, which were those animals exposed at 0.3 and 0.5 mg/L of PHMB, respectively. No test item related macroscopic observations were noted in animals exposed to concentrations up to 0.5 mg/L at terminal sacrifice (Day 14).

On the basis of this study, LC₅₀ were determined to be 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)."

Ref.: 5; 30

SCCS comment

Based on the outcome of study 2 classification as Acute tox 2, H330 (fatal if inhaled) has been proposed by RAC.

Ref.: SCCS Ref. V

According to the rules on classification of mixtures (see ECHA Guidance on the application of CLP criteria (SCCS Ref. III)) and under the assumption that all other ingredients present in the finished cosmetics products can be considered as being not acutely toxic, the acute toxicity estimate of a mixture containing 0.3 % PHBM would be 96.6, i.e. a classification as acute toxic for inhalation would not be warranted.

General SCCS comment on acute toxicity

From two acute oral toxicity studies performed with PHMB in rats, an LD₅₀ value of 1049 mg/kg bw was reported in one of the studies and LD₅₀ values of 549 mg/kg bw for males and 501 mg/kg bw for females were reported for the other study. Based on these values, PHMB can be considered of moderate acute oral toxicity; classification as Acute Tox 4 H302 (harmful if swallowed) as proposed by RAC is justified.

Two acute dermal toxicity tests have been performed. No deaths occurred up to dose levels of 5000 mg/kg bw/d. Thus it can be concluded that PHMB is not acutely toxic by the dermal pathway.

Two acute inhalation toxicity studies have been performed which were not available to the SCCS for evaluation. Recently, RAC has identified one of the studies leading to LC₅₀ values of 0.29 mg/l for males, 0.48 mg/l for females and 0.37 mg/l for males and females combined as key study thus warranting classification as Acute Tox 2 H330 (fatal if inhaled) and indicating acute inhalation toxicity. According to the rules on classification of mixtures (see ECHA Guidance on the application of CLP criteria (SCCS Ref. III)) and under the assumption that all other ingredients present in the finished cosmetics products can be considered as being not acutely toxic, the acute toxicity estimate of a mixture containing 0.3 % PHBM would be 96.6, i.e. a classification as acute toxic for inhalation would not be warranted.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Guideline:	OECD TG 404; EU B.4
Species/strain:	Rabbit / White New Zealand
Group size:	3 male animals
Test substance:	PHMB, DS6274
Batch:	PC 100-02
Purity:	reference is given to a non-attached appendix; submission states: 96 %
Vehicle:	distilled water (to moisten neat substance powder)
Dose level:	0.5 g neat substance

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Dose volume: 0.5 g neat substance moistened with 0.5 ml water
 Observation: 7 days
 GLP: yes
 Study period: 2002, report dated 2003

One animal was initially treated with the moistened test substance on three sites of the back; treated sites were then covered with a cotton gauze patch held in place by adhesive tape. Patches were successively removed after 3 minutes, 1 hr and 4 hr. After inspection of skin reactions in this animal, two further animals were treated with 0.5 g of moistened test substance which was held in place for 4 hr. After treatment periods, patches and residual materials were removed. Test sites were examined at the following time points after patch removal: 1, 24, 48 and 72 hr for evidence of primary irritation and scored according the Draize scheme.

Results:

There was no evidence of skin irritation following exposures of 3 minutes or 1 hr. After the 4-hr exposure the primary irritation index was 1.0. The mean value of 24, 48 and 72 hr for either erythema and eschar formation or oedema formation calculated for each animal tested was 1 or less. Following the 4-hr exposure well-defined erythema was noted at one treated skin site with very slight erythema at 2 treated skin sites 1 and 24 hr after patch removal. Very slight erythema was noted at all treated skin sites at the 48-hr observation and persisted at 1 treated skin site at the 72-hr observation.

Slight oedema was noted at 1 treated skin site 1 hr after patch removal with very slight oedema at the 24 and 48-hr observation. There was no skin reaction after 7 days.

Conclusion

Under the conditions of this study, PHMB was considered mildly irritating to rabbit skin.

Ref.:25; SCCS Ref. II

Guideline: preceded OECD test guideline
 Species/strain: Rabbit, White New Zealand
 Group size: 3 males, 6 females
 Test substance: Vantocil P (20 % aqueous solution of PHMB)
 Batch: BX 791/2[ADGM 1021/79]
 Purity: no information
 Vehicle: /
 Dose level:
 Dose volume: 0.5 ml
 Observation: 72 hrs / 21 day
 GLP:
 Study period: 1980

Test substance was applied to an area of approximately 6.25 cm² intact and abraded skin of the flanks of 6 female rabbits and kept for 24 hr under occlusive dressing. After patch removal the skin was examined at 24 and 72 hr for erythema and oedema and the skin irritation was assessed using the Draize method. In a separate section of the study, three male animals received the same treatment but were killed 48 (1 animal) and 72 hr (2 animals) after substance administration and skin samples were taken for histopathological examination.

Results:

Female animals: well defined to moderate erythema was observed in each animal at each skin site at 24 hr. This had subsided slightly at 72 hr. Average score was 2.3 for intact skin and 2 for abraded skin. Slight to moderate oedema was observed in all animals except one at 24 hr, but all signs of oedema subsided by 72 hr. By day 21 of the study there were signs of scabbing and healing at the site of the abrasions.

Male animals: histopathological examination of intact and abraded skin sites indicated moderate to marked acute inflammation characterised by epidermal acanthosis and a

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polymorphonuclear cell infiltrate in the superficial corium. There were also areas of focal necrosis extending slightly into the corium.

Conclusion:

The study authors concluded that the test substance was moderately irritating to intact rabbit skin, but severe irritation was observed in abraded skin.

Ref.: 57

In a barely readable test report on a skin corrosivity test performed with Vantocil P (Batch BX 791/2[ADGM 1021/79]; 20 % aqueous formulation) in White New Zealand rabbits it was stated that application of the material 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. Therefore it was concluded that a 20 % solution of Vantocil P is not corrosive to the rabbit skin

Ref.: 56

In references not available to the SCCS, the application of solid PHMB (96 %) to the skin of rabbits according to OECD 404 induced no signs of skin irritation. After dermal application of a 20 % aqueous PHMB solution moderate erythema was recorded 24 hr 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.

Ref.: 30

SCCS comment on skin irritation

Skin irritation was also observed in a 30 d repeat-dose study in rats (Ref.: 65) with a NOAEL of 20 mg/kg bw/d and in dermal carcinogenicity study in mice (Ref. 17).

3.3.2.2 Mucous membrane irritation / Eye irritation

Guideline:	OECD TG 405 (EU B.5)
Species/strain:	Rabbit, White New Zealand
Group size:	1 male animal
Test substance:	PHMB DS6274
Batch:	PC 100-02
Purity:	reference is given to a non-attached appendix; submission states: 96 %
Vehicle:	/
Dose levels:	0.1 ml neat substance
Administration:	instillation in the conjunctival sac of the right eye
GLP:	yes
Study period:	2002, test report 2003

Neat test substance was instilled into the conjunctival sac of one eye of a male rabbit, the untreated eye served as control. The eyes were not rinsed. Both eyes were examined at 1, 24, 48 and 72 hr 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.

Ref.:26

Guideline:	/
Species/strain:	Rabbit, White New Zealand
Group size:	9 females
Test substance:	Vantocil IB (20 % aqueous solution of PHMB)
Batch:	ADGM 1021/79
Purity:	no information
Vehicle:	/
Dose volume:	0.1 ml
Observation:	up to 35 days after instillation
GLP:	yes
Study period:	1981

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 hr 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. From the results of the study the authors concluded that Vantocil IB is a moderate eye irritant without irrigation and a mild irritant with irrigation.

Ref.: 75

In a recent study not available to the SCCS and performed according to OECD TG 405 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 hr after instillation and still noted at the end of the observation at day 7. It was associated with an important chemosis noted 24 hr after instillation and still noted at the end of the observation. At the corneal level, a moderate opacity was registered 1 hr after instillation and still present at the end of the observation. At the iris level, congestion was registered from the 2nd day of the test and persisted until the end of the observation.

An ulceration of the nictitating membrane and the cornea was noted from the 1st day of the test. This lesion persisted for at least 72 hr. Taking into account the severity of the reactions, the study was stopped at day 7 in accordance with the principles of animal welfare and additional animals were not treated.

Ref.:30

3.3.2.3 Respiratory irritation

Guideline/method:	/
Species/strain:	Mouse, Alpk:APfCD-1
Group size:	5 females/ group
Test substance:	a) Baquacil (20% PHMB solution), no batch stated b) Baquacil in Spa water
Dose levels:	a) 5, 50 and 200 mg/m ³ b) 10 ppm PHMB in Spa water
Control substance:	Spa water
Route:	inhalation
Exposure:	nose-only
Duration:	not mentioned

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GLP: no
Study period: 1993

Baquacil was examined for sensory irritation potential in mice after inhalation. In order to assess the sensory irritant potential of Spa water containing Baquacil, aerosol atmospheres from a dilution in pool water with final concentrations of 10 ppm PHMB at target particulate concentrations of 10000 mg/m³ were investigated in comparison. Control animals were exposed to target particulate concentrations of 10000 mg/m³ synthetic pool water. The respiration rate of the mice was measured for each target concentration using pressure plethysmography and the RD₅₀ (concentration which causes 50 % depression in respiratory rate) was calculated.

Results:

Target concentrations of 5, 50 and 200 mg/m³ PHMB corresponded to analysed concentrations of 11.7, 62.9 and 208.0 mg/m³ PHMB with median aerosol sizes (MMAD) of 2.52, 3.08 and 4.31 µm. The target concentration of 10 ppm PHMB in Spa water corresponded to analysed concentrations of 7 ppm in Spa water (particle size not determined).

The mean respiratory rate depression was 12 ± 4 %, 20 ± 7 % and 40 ± 15 % for the target concentrations of 5, 50 and 200 mg/m³, respectively and an RD₅₀ value of 264 mg/m³ was calculated. In one animal, there was a marked depression in respiratory rate, from which the animal showed little recovery. Sodium dichloroisocyanurate, another sanitising agent, which was investigated in parallel, showed an RD₅₀ value of 39.2 mg/m³, i.e. was a more potent respiratory irritant.

There was no significant difference between the respiratory depression of control animals and animals exposed to PHMB at in use concentrations in Spa water (respiratory rate depression of 19 ± 17 in PHBM containing SPA water versus 17 ± 4 in controls). The study authors concluded that Baquacil in Spa water would not be expected to induce sensory irritation.

Ref.: 80

SCCS comments

- (1) The SCCS notes that the RD₅₀-value of 264 mg/m³ is outside the investigated concentration range thus questioning the reliability of this value.
- (2) From the results of the above study, PHBM should be considered as a respiratory irritant. If present in mixtures, no classification would be warranted if the concentration of a STOT-SE 1 chemical would be ≤ 1 % in the absence of other respiratory irritants in the finished product.
- (3) The concentration of PHMB in SPA water is below the maximal intended use concentration of PHMB in cosmetics. Thus, the results obtained in SPA water are not relevant for cosmetic products.

General SCCS comment on irritation and corrosivity

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.

3.3.3 Skin sensitisation

3.3.3.1 Guinea pig maximisation test

Guideline:	OECD TG 406
Species/strain:	Guinea pig/ Alpk: Dunkin Hartley
Group size:	20 females in test group, 10 females in control group
Test substance:	PHMB, 20.2 % solution
Batch:	D4097
Purity:	not stated

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Vehicle:	deionised water
Concentration:	intradermal induction: 0.3 % of material as delivered (0.06 % a.i.) dermal induction: material as delivered (20.2 % a.i.) challenge: material as delivered (20.2 % a.i.) and 30 % of material as delivered (6 % a.i.)
Positive control:	3 % [w/v] 2-mercaptopbenzothiazole
GLP:	yes
Study period:	1992, report dated 1993

Based on the results of range finding studies, animals were intradermally injected with 0.1 ml of a 0.06 % a.i. aqueous solution with or without Freund's complete adjuvant into the shaven shoulder region of test group animals. One week later, dermal induction was performed by occlusively applying neat substance (20.2 % a.i.) in the induction sites for 48 hr. Challenge was performed by occlusive epicutaneous application of the undiluted material (20.2 % a.i.) and a 30 % solution in deionized water (6 % a.i.) to a previously untreated site for 24 hr. The application sites were scored 24 and 48 hr after removal of the patch according to the scheme.

Results:

Following challenge with 20.2 % a.i., scattered mild redness or moderate diffuse redness was observed in 18/20 of test animals at 24 hr and 16/20 test animals at 48 hr (average scores of 1.4 at 24 hr and 1.2 at 48 hr). 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 24 hr was 50 %. Following challenge with 6 % a.i., scattered mild redness or moderate diffuse redness was observed in 5/20 test animals at 24 hr and 2/20 at 48 hr (average scores of 0.3 at 24 hr and 0.1 at 48 hr) and scattered mild redness was observed only in 1 of the ten control animals at 24 hr. A strong sensitisation response was observed for the positive control.

The study authors concluded that PHMB should be considered as a moderate sensitisier.

Ref.: 27

SCCS Comment

Under the conditions of challenge with 20.2 % a.i. (50 % net frequency of response at 24 hr) the substance should be considered as strong sensitisier according to Regulation (EC) No 1272/2008 (CLP regulation).

A further guinea pig maximisation test performed according to OECD TG 406 and GLP principles was not available for evaluation to the SCCS. The study has been evaluated by RAC and reported in Ref. 30, from where the study description is cited:

"Another Guinea Pig Maximisation Test was performed according to guideline OECD 406 and GLP (Richeux, 2002c) 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 site (net frequency of response of 10%) and for one animal out of 10 at the 10% concentration site in the test group (net frequency of response of 10%). 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 (net frequency of response of 10%). No reactions were evident in the control group. Under the conditions of this study, PHMB is not considered as a dermal sensitisier according to classification criteria."

Two older guinea pig maximisation tests did not strictly adhere to OECD TG 406:

Guideline: considered comparable to OECD TG 406

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Species/strain:	Guinea pigs, Alderley Park
Group size:	20 test animals, 8 control animals, females
Test substance:	Vantocil IB (20.2 % aqueous solution of PHMB)
Batch:	[BX 791/2 (ADGM 1021)]
Purity:	no information
Vehicle:	deionised water
Concentration:	intradermal induction: 1% of material as delivered (0.2 % a.i.) dermal induction: material as delivered (20.2 % a.i.) challenge: material as delivered (20.2 % a.i.)
Positive control:	/
GLP:	yes
Study period:	report dated 1980

Animals received intradermal inductions to the clipped scapular region with 0.2 % PHMB in deionised water and Freund's complete adjuvant (FCA), 0.2 % PHMB in water and FCA plus water. 7 days later, animals received topical induction with a neat preparation of the test article (20.2 % PHMB) to the same area which was held in place for 48 hr. 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 hr (net frequency of response of 57.5 %). At 48 hr 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.:58

The possible cross-reactivity of PHMB (Vantocil IB, batch: ADGM 1021, 20 % aqueous PHMB solution) with chlorohexidine gluconate was examined in a study according to the Magnusson and Kligman method using female Dunkin Hartley guinea pigs (20 test and 8 control animals). The design was comparable to OECD 406 with the exception that no SLS was applied during the induction period. Intradermal induction was performed with 0.25 % PHMB in water and 0.25 % PHMB in water and FCA. Topical induction (one week later, 48 hr occlusive dressing) was performed with 20 % PHMB. Challenge was performed with 20 % PHMB or 0.05 %, 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 yielding a net frequency of response of 2.5 %. No cross reactivity with chlorohexidine gluconate was observed. Rechallenge with 20 % PHMB resulted in positive skin reactions in 3 out of 20 test animals. The study authors concluded that PHMB was a mild sensitisier under the conditions of this study.

Ref.:59

SCCS comment

According to CLP classification criteria, PHMB is not considered as a sensitisier based on the outcome of this study.

3.3.3.2 Buehler test

Guideline:	considered comparable to OECD TG 406
Species/strain:	Guinea pigs, Alderley Park
Group size:	10 test animals, 10 control animals, females
Test substance:	Vantocil IB (20 % aqueous solution of PHMB)
Batch:	[BX 791/2 (ADGM 1021)]
Purity:	no information
Vehicle:	
Concentration:	topical induction and challenge: 10 % of material as delivered (2 % a.i.) Rechallenge: 20 %, 10 % and 1 % of material as delivered (4, 2 and 0.2 % a.i.)

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Positive control: /
 GLP: yes
 Study period: report dated 1980

For induction, animals received topical application of 0.4 ml of test substance (2 % a.i.) or vehicle to the clipped scapular region. The induction site was occlusively covered and the dressing was held in place for 6 hr. Induction with 2 % a.i. was repeated for 10 6-day treatments within 3 weeks. Challenge exposures (2 % a.i.) of 6 hr were performed two weeks after the last induction exposure and reactions were scored 24 and 48 hr after patch removal.

Challenge of test and control animals resulted in signs of faint erythema in 6/10 test animals and no erythema in control animals at 48 hr. Rechallenge with 4 % a.i. resulted in faint to moderate erythema in 8/9 test animals and faint erythema in 3/10 controls. Rechallenge with 2 % a.i. resulted in faint erythema in 3/10 test animals and no erythema in controls. Rechallenge with 0.2 % a.i. resulted in no response in test and control animals. The study authors considered the test substance as 2 % a.i. as a moderate sensitisier under the conditions of this study. A challenge dose-response was postulated.

Ref.: 58

SCCS comment

According to CLP classification criteria, PHMB should be considered as a moderate sensitisier based on the outcome of this study.

Guideline: considered comparable to OECD TG 406
 Species/strain: Guinea pigs, Alderley Park
 Group size: 10 males and 10 females per study group
 Test substance: Vantocil IB (20 % aqueous solution of PHMB)
 Batch: [BX 791/2 (ADGM 1021)]
 Purity: no information
 Vehicle: water
 Concentration: see table
 Positive control: /
 GLP: yes
 Study period: report dated 1983

The effect of variation of induction and challenge concentrations of PHMB as postulated in the earlier study was further investigated in a study investigating a variety of induction and challenge concentration by using the Buehler protocol.

The concentrations used for induction and challenge exposures as well as the results are given in table 3.

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Table 3: overview on responses to PHMB under various induction and challenge conditions

Induction concentration [%]	Challenge concentration [%]	Re-challenge concentration [%]	Response in test animals	Response in control animals	Net response [%]	Sensitiser [Potency CLP criteria]
0.3	0.3/0.15/ 0.075/0.03	/	/	/	0	no
0.8	0.8/0.4/ 0.2/0.08	/	/	/	0	no
1.3	1.3/0.65/ 0.325/0.13	/	/	/	0	no
1.8	1.8/0.9/ 0.45/0.18	/	/	/	0	no
2.0	2.0	/	1/10	0/10	10	no
		2.0	0/10	0/10	0	
1.2	1.2	/	0/10	0/10	0	no
		1.2	0/10	0/10	0	no
		15	3/10	0/10	30	moderate
5	15	/	6/8	0/10	75	strong
		2	6/8	0/10	75	strong
		1.2	4/8	0/10	50	moderate

The study authors concluded that the threshold for eliciting sensitisations in guinea pigs is approximately 1 %.

Ref.: 60; 30

SCCS comments

Under the conditions of this study, PHMB is a moderate to strong sensitisier at concentrations above 1.2 %.

General SCCS comment on sensitization

Based on various guinea pig maximization- and Buehler tests, PHMB can be considered as a moderate to strong sensitisier in animals. The threshold for eliciting sensitisations in guinea pigs is approximately 1 %. The SCCS notes that classification as Skin Sens 1B H317 according to CLP criteria is currently proposed (Ref. 30).

3.3.4 Dermal / percutaneous absorption

Guideline:	OECD TG 428
Species/strain:	Human skin
Number of donors:	Abdomen skin from 7 donors (33-49 years)
Test system:	Split-thickness skin samples (380 – 400 µm), static diffusion cells, exposure area 3.14cm ² , Receptor chamber volume 10 ml
Membrane integrity:	membranes with a resistance < 10 kΩ (electrical barrier resistance test) were excluded
Test substance:	PHMB
Batch (labelled test substance):	99BDR99, [¹⁴ C]-PHMB, specific activity: 38.9 mCi/g
Purity (labelled test substance):	9.84 % radiochemical purity (HPLC)
Batch (unlabelled test substance):	09GR24243581, 19.2% aqueous PHMB
Purity (unlabelled test substance):	no information
Test item:	Oil/Water emulsion with labelled and non-labelled PHMB
Dose level:	2.06 mg/cm ² , Total PHMB applied 18.8 µg
Receptor fluid:	phosphate buffered saline (PBS)
Method of Analysis:	liquid scintillation counting
GLP:	yes (report not signed by the study QA responsible person)
Study period:	2014

Split-thickness skin samples (380 – 400 µm) from 7 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 about 0.3 % [w/w] in a representative cosmetic formulation. A temperature of 32 ± 1°C was maintained in the system, the application rate was 2.0 and 2.11 mg/cm² (target: 2.0 mg/cm²). Receptor fluid was collected prior to dosing and 0.5, 1, 2, 4, 8 and 24 hr after dosing. At the last sampling point, skin samples were washed, dried and removed from the apparatus in order to remove stratum corneum by 20 tape stripplings. Epidermis and dermis were then heat separated and all skin samples were analysed by liquid scintillation counting.

Results:

Only 12 samples from 5 donors (from a total of 19 samples from 7 donors) could be used for evaluation due to processing errors and one sample being considered as an outlier. The mean mass balance was 97.60 % of the applied dose at 24 hr post dose. At the end of the 24 hr exposure period, 49.15 % was washed off. A further 0.54 % of the applied dose was removed from the donor chamber wash leading to a total dislodgeable dose of 49.69 % of

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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 were not considered to be dermally absorbed. 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 %). 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. An overview on the results of the dermal absorption study is given in table 4.

Table 4: results of the *in vitro* dermal absorption study performed by Toner, 2014.

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 + <i>stratum corneum</i> + 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

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(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

SCCS comment

The study was performed according to OECD TG 428 and SCCS basic requirements for dermal penetration studies and was in line with GLP principles. Despite some processing errors and an outlier, and despite the fact that the study is still in the state of a draft report, it is considered acceptable for determination of dermal absorption of PHMB. The SCCS notes, that 20 tape strippings were used to remove stratum corneum, which is a high number. Normally, not more than 5 tape strippings should be used to remove stratum corneum. Thus, it cannot be excluded that some absorbable amounts of PHMB were removed by the high number of tape strippings used. The SCCS further notes that no information was given on the position of radiolabel within the molecule.

The SCCS also notes that the applicant intends to defend the safety of PHMB for all types of cosmetics products, whereas dermal penetration was only investigated in one type of cosmetic formulation (oil/water emulsion). Based on the variability observed in the study, the unusual high number of tape strippings used and the fact that dermal penetration was only investigated in one type of cosmetic formulation, the mean + 2 SD will be taken for MOS calculation , i.e. $3.49 + 4.16\% = 7.65\%$ or $207.94 + 247.54 \text{ ng equiv./cm}^2 = 455.48 \text{ ng equiv./cm}^2$.

Reference: Toner, 2014

Guideline:	/
Species/strain:	Human skin
Membrane integrity:	membranes with a calculated resistance < 10 kΩ were excluded
Number of donors:	not stated
Method:	analysis of receptor fluid in glass diffusion cells
Test substance (unlabelled):	PHMB from Zeneca Inc. Dighton, Mass., USA; 20.2 % aqueous solution
Batch:	no data
Purity:	no information
Test substance (labelled):	[¹⁴ C] PHMB
Radiochemical reference no.:	4602
Specific activity:	1.85 GBq/732 mg
Dose volume:	10 and 200 µl/cm ²
Receptor fluid:	distilled water
Method of Analysis:	liquid scintillation counting
GLP:	yes

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Study period: 1996

Epidermal membranes from human skins stored frozen (no further information on donors, skin location, number of individual donors) were used for the study. Skin samples were mounted on glass diffusion cells (exposure area: 2.54 cm²) and placed in a water bath maintained at 30 ± 1 °C. Only membranes with a calculated resistance > 10 kΩ were used. Admixture of labelled compound to unlabelled compound was performed to achieve activities between 3 x 10⁶ and 4 x 10⁹ dpm/ml. Nominal concentrations of 200.0, 20.0 and 2.0 g/l were applied at 10 µl/cm² and left unoccluded whereas the concentration of 200 g/l was also applied at 200 µl/cm² and kept occluded. At recorded time intervals, 250 µl samples of receptor fluid were taken for analysis; the volume removed from the receptor chamber was replaced by addition of the same volume of receptor fluid. The total observation period was 96 hr.

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Results:

Table 4: *in vitro* dermal absorption of [¹⁴C] PHMB in human skin

Test condition [concentration, applied rate, occlusion]	Time period [h]	Absorption rate [µg/cm ² /hr]	Absorption percentage [%]
201 g a.i./l 200 µl/cm ² occluded [n=4]	0 - 24 0 - 96	0.110 ± 0.044 0.088 ± 0.033	0.001 0.021
197 g a.i./l 10 µl/cm ² unoccluded [n=5]	0 - 24 0 - 96	0.009 ± 0.003 0.007 ± 0.002	0.012 0.036
20.9 g a.i./l 10 µl/cm ² unoccluded [n=8]	0 - 24 0 - 96	0.005 ± 0.001 0.003 ± 0.001	0.059 0.146
1.93 g a.i./l 10 µl/cm ² unoccluded [n=8]	0 - 24 0 - 96	0.001 ± 0.001 < 0.001 ± < 0.001	0.088 0.129

SCCS comment

There was no information on skin donors, numbers of individual skin donors, location from which skin sample was taken, skin thickness. Recovery was not reported and only results from receptor fluid but not from other compartments (e.g. skin wash, amounts remaining in epidermis) are reported. Thus realistic dermal absorption rate of PHMB cannot be derived from this study.

Ref.: 19

Guideline:	/
Species/strain:	Human skin
Membrane integrity:	membranes with a calculated resistance < 10 kΩ were excluded
Number of donors:	not stated
Method:	analysis of receptor fluid in glass diffusion cells
Test substance (unlabelled):	PHMB from Zeneca Inc. Dighton, Mass., USA; 20.2 % aqueous solution
Batch:	no data
Purity:	no information
Test substance (labelled):	[¹⁴ C] PHMB
Radiochemical reference no.:	1581
Specific activity:	1.4 MBq/mg
Dose volume:	200 µl/cm ²

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Receptor fluid: distilled water
 Method of Analysis: liquid scintillation counting
 GLP: yes
 Study period: 1998

Epidermal membranes from human skins stored frozen (no further information on donors, skin location, number of individual donors) were used for the study intended to investigate skin penetration at elevated temperature (spa conditions). Skin samples were mounted on glass diffusion cells (exposure area: 2.54 cm²) and placed in a water bath maintained at 40 ± 1 °C. Only membranes with a calculated resistance > 10 kΩ were used. Admixture of labelled compound to unlabelled compound was performed to achieve activities between 3 x 10⁸ dpm/ml. A 20 % dosing solution was warmed to 40°C and applied at 200 µl/cm² to two groups of skin membranes. After application, one group was occluded for 0.5 hrs and then washed with 3 % Teepol® and distilled water and the kept unoccluded until 24 hr after application. The other group of membranes was kept occluded for 24 hr after substance application. At recorded time intervals, 250 µl samples of receptor fluid were taken for analysis; the volume removed from the receptor chamber was replaced by addition of the same volume of receptor fluid. The total observation period was 96 hrs.

Results:

Table 5: *in vitro* dermal absorption of [¹⁴C] PHMB in human skin

Test condition [concentration, applied rate, occlusion]	Time period [hr]	Absorption rate [µg/cm ² /hr]	Absorption percentage [%]
200 g a.i./l 200 µl/cm ² occluded for 0.5 hr, then unoccluded for 23.5. hrs [n=6]	0 – 24	< 0.002 ± < 0.001	< LOQ
200 g a.i./l 200 µl/cm ² occluded [n=5]	0 – 24	0.118 ± 0.012	0.007

There was no information on skin donors, numbers of individual skin donors, location from which skin sample was taken, skin thickness. The volume of test solution applied i.e. 200 µl/cm² was much higher than the recommended 10 µl/cm². Recovery was not reported and only results from receptor fluid but not from other compartments (e.g. skin wash, amounts remaining in epidermis) are reported. The temperature was higher than recommended. Thus realistic dermal absorption rate of PHMB cannot be derived from this study.

Ref.: 20

Guideline: /
 Species/strain: epidermis from human abdominal skin
 whole skin from flank and dorsum of Wistar-derived

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Membrane integrity:	Alderley-Park rats (male and female) permeability constants (tritiated water) $> 1.3 \times 10^{-3}$ cm/hr (human skin) and $> 1.5 \times 10^{-3}$ cm/hr (rat skin) excluded
Group size:	/
Method:	wet and "dried on" absorption experiments
Test substance (unlabelled):	20 % aqueous PHMB
Batch:	test substance code Y0156/001/002
Purity:	no information given
Test substance (unlabelled):	¹⁴ C- PHMB, slightly higher than 20% aqueous solution
Batch:	CTL Radiochemical Card 878
Dose volume:	Specific activity: 0.88 mCi/ml
Receptor fluid:	1 ml
Method of Analysis:	sterile physiological saline
GLP:	LSC
Study period:	yes
	1982

Absorption from aqueous solutions could not be measured below 0.4 % ¹⁴C PHMB.

Human and rat skin samples were mounted on glass diffusion cells. After checking integrity, 1 ml of PHBM solution (17.6 µCi) was added to the donor chamber in the wet experiments. At least 2 x 25 µl receptor fluid samples were taken daily for up to 15 days. In dried-on experiments, skin was exposed to atmosphere overnight before the addition of 36 µl 20 % ¹⁴C PHMB (31.7µCi). 25 µl samples were taken daily until slow absorption rate became apparent.

Furthermore, an uptake experiment was performed in which 2 cm² skin disks were taken into "bathing" solutions of different PHMB concentrations. After an equilibration phase of 5 days, skin samples were weighed, dissolved and partition coefficients were determined. Under dried-on conditions, absorption of PHBM through human skin was not measurable, which might in part be due to the limits of detection (absorption from aqueous solutions could not be measured below 0.4 % ¹⁴C PHMB). Steady state of absorption rates achieved under wet conditions are presented in the table 6. The LOQ and the low absorption rate complicated the determination of a permeability constant; however, a value of 5×10^{-6} cm hr⁻¹ was suggested by the study authors.

Table 6: *in vitro* dermal absorption of [¹⁴C] PHMB in rat and human skin

Skin sample	PHBM concentration [%]	Absorption rate [ng cm ⁻² hr ⁻¹]
Human epidermis	0.4	8.13
	1.4	22.8
	5	350.0
	20	1005.0
Rat whole skin	0.4	131.0
	20 (early phase)	3695.0
	20 (late phase)	11940.0

SCCS comment

Skin samples are poorly described; there is no information on number of donors. Number of skin samples analysed per donor is also not stated. The surface area exposed to the test item is not known. Recovery was not reported. The study was not performed according to current guidelines. Results of this study cannot be considered to derive a realistic dermal absorption rate of PHMB.

Ref.:28

There also exists a previously performed comparative penetration study of lower reliability. Radiolabelled PHMB ($[^{14}\text{C}]$ -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 hr at one-hr intervals and thereafter 3 samples were taken daily during a 8 hr period 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 hr, 1 ml DMSO was added. The radioactivity was measured by means of liquid scintillation counter. 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 hr and this penetration rate was from 0.11 % up to 0.81 % after adding DMSO.

Ref.: 61

SCCS comments

This study preceded OECD test guidelines and GLP. Human skin samples were not properly described and only 4 skin samples were used. Skin integrity was not tested. The standard deviations of the absorption rates were not reported. The dermal absorption rate obtained from this study cannot be considered for the calculation of MoS.

General SCCS comment on percutaneous penetration

Four studies on percutaneous penetration of PHMB were not in compliance with SCCS requirements, qualities of the studies were compromised by poor sample description and the studies have not been performed with representative cosmetic formulations containing 0.3 % PHMB. After the commenting period which followed the publication of SCCS/1535/14 on 18 June 2014, a draft of a new *in vitro* percutaneous absorption study was provided by the applicant.

The new dermal penetration study was performed according to OECD TG 428 and SCCS basic requirements for dermal penetration studies and was in line with GLP principles. Despite some processing errors and an outlier and despite the fact the study is still in the state of a draft report it is considered acceptable for determination of dermal absorption of PHMB.

The SCCS notes, that 20 tape strippings were used to remove stratum corneum, which is a high number. Normally, not more than 5 tape strippings should be used to remove stratum corneum. Thus, it cannot be excluded that some absorbable amounts of PHMB were removed by the high number of tape strippings used. The SCCS further notes that no information was given on the position of radiolabel within the molecule.

The SCCS also notes that the applicant intends to defend the safety of PHMB for all types of cosmetics products, whereas dermal penetration was only investigated in one type of formulation (oil/water emulsion). Based on the variability observed in the study, the unusual high number of tape strippings used and the fact that dermal penetration was only investigated in one type of cosmetic formulation, the mean + 2 SD will be taken for MOS calculation, i.e. $3.49 + 4.16\% = 7.65\%$ or $207.94 + 247.54 \text{ ng equiv./cm}^2 = 455.48 \text{ ng equiv./cm}^2$.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (short-term) toxicity

Oral

Guideline:	/ (range-finding study for 2-year drinking water study)
Species/strain:	Rat, Alpk:APfSD
Group size:	8/sex/dose
Test substance:	PHMB, 20 % aqueous solution
Batch:	D4097
Purity:	no details given
Vehicle:	sterilised water
Dose levels:	0, 0.1, 0.5, 1.0, 2.0 mg/ml
Route:	oral
Administration:	drinking water
Duration:	28 days
GLP:	no statement
Study period:	1991, report dated 1992

The stability of the test sample in drinking water was determined prior to start. Clinical observations were recorded daily, bodyweights and food and water consumption were recorded throughout the study. After the 28-day treatment period, surviving animals were killed, subjected to a gross post-mortem examination; liver and kidney weights were recorded and blood samples were taken.

Results:

One male and one female animal receiving the highest dose were killed on day 4 due to bodyweight loss and deterioration of clinical condition. Dose-related loss in bodyweight/body weight gain and reduced water and/or food consumption predominantly occurring during the first days of treatment (and recovery thereafter) were considered as a palatability effect. Further treatment-related findings consisted of increases in plasma cholesterol levels and ALP and AST activities at the highest dose tested, increased liver weight at 1 mg/ml and decreased liver weight at 2 mg/ml and a dose-related increase in kidney weight at all dose levels. No NOAEL could be derived from the study. A highest dose level of 1.0 mg/ml was recommended for the 2-year drinking water study. 0.1 mg/ml should be considered as LOAEL based on the results from this study.

Ref.: 47

Guideline:	/ (range-finding study for 2 year drinking water study)
Species/strain:	Mouse, C57Bl/10JfAP/Alpk
Group size:	10/sex/dose
Test substance:	PHMB, 20 % aqueous solution
Batch:	D4097
Purity:	no details given
Vehicle:	sterilised water
Dose levels:	0, 0.1, 0.3, 0.6, 1.2 mg/ml
Route:	oral
Administration:	drinking water
Duration:	28 days
GLP:	no statement
Study period:	1991

The stability of the test sample in drinking water was determined prior to start. Individual clinical observations were recorded daily. Individual bodyweights and food consumption per cage (5 animals/cage) were recorded daily for the first week and weekly thereafter. After 28 days of treatment, all surviving animals were killed and subjected to gross post mortem

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examination. Liver and kidney weights were recorded and blood samples were obtained from each animal.

Results:

One male animal of the 0.3 mg/ml group was 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 were considered to be due to palatability of the substance. Changes considered to be treatment-related included a decrease in plasma ALT activity and a decrease in liver weight for males given 0.6 and 1.2 mg/ml and were most probably associated with the poor nutritional status. As effects on bodyweights and water consumption were present at all dose levels, a NOAEL could not be derived. A highest dose level of 0.3 mg/ml was recommended for the 2-year drinking water study. 0.1 mg/ml should be considered as LOAEL based on the results from this study.

Ref.: 48

Dermal

Guideline:	/ (similar to OECD TG 410)
Species/strain:	Rat, Alpk:APfSD (Wistar-derived)
Group size:	5/sex/dose
Test substance:	Vantocil P (20.2 % aqueous solution of PHMB)
Batch:	ASG9112994
Purity:	impurities < 0.76 %
Vehicle:	/
Dose levels:	0, 20, 60, 200 mg PHMB/kg
Route:	dermal
Administration:	occlusive, 6 hr /day
Duration:	30 days/ 21 applications
GLP:	yes
Study period:	1993

21 occlusive 6-hr test substance applications were made to the shorn back during a period of 30 days. On days of dosing, oral uptake was prevented by fitting the animals with plastic collars after removal of the dressing to prevent oral uptake. At termination of treatment, blood samples were taken for haematology and clinical pathology and gross pathology was performed; testes, kidneys and livers were weighed, selected organs were preserved.

Results

There were no mortalities and no overt clinical signs of toxicity up to the highest dose tested. 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. 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. 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.

Ref.: 65

In an earlier study, comparable results have been obtained: PHMB (Vantocil IB) was applied to a group of six female albino rabbits daily to their shorn backs for 23 hr 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 livers, adrenals, kidneys, spleens, ovaries, uteri, hearts, thymuses and lungs were examined histopathologically.

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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: Elks and Hope, 1972).

Ref.: 32

Inhalation

Guideline:	OECD TG 412
Species/strain:	Rat, Alpk:APfSD (Wistar-derived)
Group size:	5/sex/dose (group)
Test substance:	PHMB, 19.2 % [w/w] aqueous solution
Batch:	BX 6142
Purity:	HMD < 0.1 %; HMBDA 0.1 %
Dose levels:	0; 0.025; 0.25; 2.5 µg/l [mg/m ³]
Vehicle:	deionised water
Route:	inhalation
Administration:	nose-only: 6 hr/day; 5 days/week
Duration:	28 days (13 week recovery for satellite groups at 0; 0.025; 2.5 µg/l)
GLP:	yes
Study period:	2003 - 2004, report dated 2006

The study was preceded by two 5-day range-finding studies in rats. In these studies, dose-dependent histopathological findings in lung and larynx have been observed, indicative of respiratory irritation.

Animals were exposed as indicated. Target concentrations of aqueous solutions of 0 (control), 0.025, 0.25 or 2.5 mg/m³ corresponded to PHMB concentrations of 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). 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, selected tissues were weighed (adrenal glands, ovaries, epididymis, spleen, kidneys, testes, liver, thymus, lungs (with trachea attached but larynx removed), brain, heart, uterus and cervix). Tissues were almost completely taken and processed for subsequent histopathology.

Results

There were no treatment-attributable deaths and clinical signs up to 2.5 mg/m³ (clinical observations during exposure and post-exposure were attributed to the restraint nose-only conditions). Body weights were lower than for controls in males exposed to 0.25 mg/m³ or 2.5 mg/m³. Following cessation of exposure there was some recovery in body weight 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 elevated for males and females exposed to 2.5 mg/m³ and thymus weights were slightly elevated in males only at 2.5 mg/m³. No macroscopic treatment-related findings were observed at post-mortem examination.

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. Comparable findings were absent 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. The severity of pneumonitis was slightly reduced at the end of the recovery period. The higher thymus weight for males only

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exposed to 2.5 mg/m³, in the absence of any histopathological changes, was considered to be of unknown toxicological significance.

PHMB exposure to concentrations of 0.257 or 2.47 mg/m³ resulted in 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. Some bodyweight 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 2.47 mg/m³. A NOAEL of 0.0239 mg/m³ was derived from the study.

Ref.: 76, 77, 78

Guideline:	/ (precedes guideline program)
Species/strain:	Rat, Alderley Park SPF albino strain
Group size:	4/sex/dose
Test substance:	Vantocil IB (20 % aqueous solution of PHMB)
Batch:	Std Sample BX 16, 17, 18, 19 ADOH. 4848/73
Purity:	not stated
Vehicle:	
Dose levels:	0.025; 0.25; 2.75, 12.5 and 26 mg/m ³
Route:	inhalation
Administration:	snout-only: 6 hr/day; 5 days/week
Duration:	3 weeks
GLP:	/
Study period:	1976

Animals were exposed as indicated to atmospheres of respirable particles (MMAD < 7 µm). Body weight, food and water intake and signs of gross toxicity were checked daily. After the last exposure, blood and urine samples were obtained, animals were killed and selected tissues (lung, trachea, thymus, liver, kidneys, adrenals, spleen, heart, gonads, epididymes, uteri) were taken for histopathological examination.

Results:

There were no signs of toxicity at the lowest dose tested. At 0.25 mg/m³ one animal died after the 13th exposure and animals did not gain weight or even lost weight. The experiment was terminated after 13 exposures. Animals showed signs of moderate nasal irritation and tachypnoea. There were significant amounts of methaemoglobin in all animals (5, 4 and 4% in males and 3, 7, 5 and 3% in females). Histopathological examination of stained sections revealed slight to moderately severe pneumonitis. There was also evidence of accompanying resolution of the lung lesions in all the affected animals. The thymuses of 3 male and 3 female rats from the test group showed reduction in the cortical thickness and depletion of lymphocytes. Patchy loss of cilia in the tracheal epithelium was observed in three animals. The testis of one male showed degeneration of a few seminiferous tubules. At 2.75 mg/m³ animals showed signs of nasal irritation and dyspnoea and dramatic body weight loss after the 4th exposure. The experiment was terminated after the 6th exposure. There were significant increases in methaemoglobin in all animals. Histopathological examination of tissues revealed a moderate to severe pneumonitis. The thymus glands showed severe depletion of lymphocytes and loss of normal architecture. Exposure to 12.5 mg/m³ resulted in severe nasal irritation and dyspnoea. During the first three days of exposure, all animals lost weight and their intake of food and water was very low. One female rat died towards the end of the fourth exposure and the remainder died overnight. Exposure of rats to 26.0 mg/m³ resulted in severe nasal irritation and dyspnoea. During the first three days of exposure all animals lost weight and their intake of food and water was very low. As in the test with 12.5 mg/m³ exposure, one female rat died towards the end of the fourth exposure and the remainder died overnight. A NOAEL of 0.025 mg/m³ was derived from this study.

Ref.: 15

Comment SCCS on repeat-dose inhalation studies

Based on the severity of the effects caused by inhalation of PHMB, the absence of reversibility of inflammation in the respiratory tract and the very low doses causing these effects, the RAC conclusion is supported which states that according to CLP classification as STOT RE 1 H372 (causes damage to the respiratory tract through prolonged or repeated exposure by inhalation) is warranted.

According to the procedures of classification of mixtures, classification with respect to STOT-RE is not warranted if the substance classified as STOT-RE 1 is present at concentrations below 1% in the mixture. See ECHA Guidance on the application of CLP criteria (SCCS Ref. III).

3.3.5.2 Sub-chronic (90 days) toxicity (oral)

The subchronic toxicity of Vantocil P (20.2 % aqueous PHMB solution, Batch D4097) was investigated in 90 d dietary studies in mice (C57Bl/10JfCD-1) and rats (Alpk:APfSD, Wistar derived; 12/sex/dose with 4 animals/sex/group used for interim sacrifices at 29 days) intended as range-finders for chronic toxicity studies. The studies were in accordance with GLP but not strictly in accordance with OECD TG 408. Dose levels in rats were 0, 1000, 2000, 4000, 6000 ppm a.i. (corresponding to about 0, 83.9, 171.5, 373.0, 556.1 mg/kg bw/day a.i. in males and 92.3, 192.9, 409.8, 617.4 mg/kg bw/day a.i. in females). In mice, dose levels were 0, 1000, 2000, 4000 ppm a.i. (corresponding to about 0, 162, 328, 736 nmkg/kg bw/day a.i. in males and 0, 224, 445, 963 mg/kg bw/day a.i. in females) and 6000 ppm a.i. (terminated due to high mortality).

Results in rats:

Dietary administration of PHMB led to a treatment-related reduction in body weight at \geq 2000 ppm accompanied by clinical-chemical indication of poor nutritional state. From 2000 ppm there were findings in some haematological parameters, notably increased haemoglobin and haematocrit in males. 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 were recorded at day 29 at the highest dose. At terminal kill, a clear treatment-related increase in kidney weight was apparent for males at 4000 or 6000 ppm, but histopathology of kidneys to assess the toxicological significance of these weight changes was not performed at terminal kill. Treatment-related increases in plasma alkaline phosphatase, alanine transaminase and/or aspartate transaminase activities were seen at all dose levels for males and for females which received 1000, 2000, 4000 or 6000 ppm at interim and/or terminal kill. As only limited histopathological examination was performed, the toxicological significance of macroscopic changes in the gastrointestinal tract, liver and hepatic lymph node in some terminal kill animals at 6000 ppm could not be assessed. It was concluded that a dose level of 2000 ppm would be suitable for use as the high dose level in a long-term dietary study. The authors considered 1000 ppm (corresponding to 83.9 mg/kg bw/d in males and 92.3 mg/kg bw/d in females) as NOAEL.

Results in mice:

There were treatment-related effects on initial food consumption in males. Reduced body weights were noted in males at 2000 ppm and marked effects on body weight gain were observed in males and females at 4000 ppm. The highest dose caused marked toxicity leading to termination of this group by day 11. There were no treatment-related effects on liver and kidney weights and no gross or histopathological findings. 4000 ppm was considered as the highest dose to be applied in a long-term feeding study. The authors considered 1000 ppm (corresponding to 162 mg/kg bw/d in males and 224 mg/kg bw/d in females) as NOAEL of this study.

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Ref.: 50, 51

An older, non-guideline compliant 90-day dietary study was performed with antibacterial 9073 (25 % aqueous solution of PHMB; Batch WEM/G/680) in Alderley Park Wistar Rats at dose levels of 0, 625 and 1250 ppm a.i. There was no mortality at any dose level. The dietary administration of 1250 ppm led to a retardation of body weight gain most likely due to reduced food consumption due to decreased palatability. At this dose level, deposits of an iron-pigment in the liver (in liver cells and in Kupffer cells) was noted in individual female rats. The dose level of 625 ppm was tolerated without any finding.

Ref.: 41

A non-guideline 90-day dietary study as a range-finder was performed with antibacterial 9073 (25 % aqueous solution of PHMB; Batch WEM/G/680) in Beagle dogs (inbred strain from Alderley Park, Cheshire). Groups of 4 animals/sex/dose received 0, 1375 or 2750 ppm a.i. as dietary admixture. Animals were weighed weekly. At the end of the study, clinical-chemical parameters were determined, a full post mortem examination was performed and the weight of selected tissues was determined. There was no mortality or no sign of clear systemic toxicity at any dose level. The only finding observed was a slight reduction in neutrophils of questionable relevance.

Ref.: 42

In a poorly reported 90 d drinking water study performed with a 20 % aqueous solution of PHMB (batch D4097) in mice (C57BL/10JfAP/Alpk), only the effect on body weight and macroscopy was investigated. A control and a dose group were used consisting of 10 males and females, respectively. The treatment group received 0.1 mg/ml test substance in the first week, 0.3 mg/ml in the second week and 0.3 mg/ml from the third week until study termination. Administration of PHMB caused a reduction in bodyweight gain of treated animals and a dose-related reduction in water consumption. There were no treatment-related macroscopic post-mortem findings.

Ref.: 49

3.3.5.3 Chronic (> 12 months) toxicity

Guideline:	considered mainly in accordance with OECD TG 452
Species/strain:	Dog/Beagle
Group size:	4/sex/dose
Test substance:	PHMB ASG9112994, 20.2 % aqueous solution
Batch:	D40987
Purity:	impurities < 0.76 %
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
Administration:	diet
Exposure:	daily for 1 year
GLP:	no statement
Study period:	1993 – 1994, report dated 1995

Dose levels were selected based on the findings of two short-term range-finding studies. Animals received PHMB as dietary admixture. The high dose of 4500 ppm was reduced to 3000 ppm from weeks 11/12 onward due to unexpectedly severe reactions (see results) leading to intercurrent killing of 3 high dose males. The animals were examined for mortality and clinical signs including behaviour. Body weight and food consumption were determined at regular intervals. Blood samples for haematology 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 subjected to a complete necropsy. Adrenal glands, brain, epididymis, kidneys, liver, testes and thyroid glands were weighed. A

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comprehensive number of organs and tissues were processed and examined by histopathology.

Results

High dose males exhibited unexpected signs of toxicity including marked reddening/peeling of scrotal skin, loss of appetite, body weight loss and/or indications of liver impairment in the form of elevated plasma alanine transaminase and/or aspartate transaminase activities. Following reduction of the high dose level to 3000 ppm, one female was killed after showing clinical signs of toxicity. For animals surviving to termination, treatment related red/brown staining of coat, predominantly paws and hocks, present in one female receiving 300 ppm and in all animals at 1500 or 3000 ppm were considered of no toxicological relevance as there were no associated histopathological changes. Plasma cholesterol levels were reduced for the surviving animals receiving 3000 ppm whereas plasma alanine transaminase and/or aspartate transaminase activities were elevated for the surviving high dose male, for 2/3 females receiving 3000 ppm and for one female each at 300 or 1500 ppm. Low testes weight was also apparent for the surviving male at 3000 ppm.

Treatment-related histopathological findings were present in the skin (dermatitis of the scrotum, chin and limbs) as well as in the liver, kidney (males only) and testes 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. The study authors considered 1500 ppm (54 mg/kg bw/d) as NOAEL of the study.

Ref.: 52, 81, 82

SCCS comment

SCCS is aware that RAC and ANSES agreed to this NOAEL.

Guideline:	Considered comparable to OECD TG 453
Species/strain:	Rat/Alpk:APfSD (Wistar derived)
Group size:	64/sex/dose (12 animals/sex/dose used for interim kill)
Test substance:	Vantocil P ASG9112994, 20.2 % aqueous solution
Batch:	D4097
Purity:	impurities < 0.76 %
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
Administration:	diet
Exposure period:	104 weeks
GLP:	yes
Study period:	1992 – 1994, report dated 1996

Animals received diet containing PHMB as indicated and were examined daily for mortality, clinical signs and behaviour. Body weight and food consumption were determined at regular intervals, ophthalmology was performed. Blood samples for haematology 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. Animals were subjected to complete necropsy when killed, a comprehensive number of organs and tissues were processed and examined by histopathology.

Results:

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

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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 hepatitis in males at this dose level were considered as a mild effect in the liver.

The pathological examination showed no non-neoplastic and no 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 tumour 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 and confirmed by a further independent expert, it was concluded that the overall weight of evidence indicated that the slightly higher number of high dose animals having vascular neoplasms of the liver is not associated with the dietary administration of PHMB. RAC concluded that evidence from this rat study is not sufficient to conclude a clear treatment-related effect with respect to vascular tumours.

Based on the findings of this study, a NOAEL of 2000 ppm PHMB in diet corresponding to 36 and 45 mg/kg bw/d was derived for male and female animals, respectively.

Ref.: 53, 13, 21, 30

Summary repeat-dose toxicity

Repeat-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 (rat, mouse, Beagle dog).

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 (ALP, ALT, ASP). However, interpretation of the dietary subchronic studies was hampered by limited histopathologic examination.

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 NOAELs of 36 and 45 mg/kg bw/d were derived for 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 hepatitis. Further, the incidence of haemangiosarcomas was increased at the highest dose tested.

From the latter study, the SCCS will take the NOAEL of 36 mg/kg bw/d for MoS calculation /safety evaluation.

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline: OECD TG 471

Test system: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538

Replicates: Triplicate plates

Test substance: Vantocil IB, 19.6 % aqueous solution

Batch: Bx2125

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Purity: /
 Solvent: DMSO
 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
 Treatment: plate incorporation assay with minor modifications
 GLP: yes
 Study period: 1988, report dated 1989

PHMB was investigated in the bacterial reverse mutation test with and without metabolic activation by S9-mix prepared from Aroclor 1254 induced liver from Alderley Park rats. In a range-finding experiment, significant toxicity was observed at concentrations > 200 µg/plate in TA100 ± S9. Therefore, 500 µg/plate was used as top concentration in the 2 main experiments. For control purposes, the negative, vehicle (DMSO) and positive controls (+ S9: N-methyl-N-nitro-N-nitrosoguanidine (MNNG) for TA 100 and TA 1535, Daunorubicin (DR) for TA98, Acridine mutagen ICR191 for TA1537 and 4-nitro-O-phenylenediamine (4-NPD) for TA 1538; -S9: 2-aminoanthracene (2-AA) for all strains) were also investigated.

Results:

In two separate experiments, PHMB did not induce significant reproducible increases in revertant colonies in TA100, TA1535, TA1537 and TA1538 in the absence or presence of S9 mix. In TA98, there were no increases in revertant colonies without S9 mix, but in the presence of S9 mix, slight responses (2.1 x background) were observed which were not reproducible and associated with an unusual low negative control for this strain. Therefore it was concluded that under the conditions of this assay PHMB was non-mutagenic in the bacterial gene mutation test.

Ref.: 14

An earlier bacterial reverse mutation test which preceded OECD test guidelines and GLP has been performed with Vantocil IB (20 % aqueous solution), batch AGDM 2253/77, in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 ± S9 mix. PHMB was toxic at a concentration 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.

Ref.: 43

A further bacterial reverse mutation test performed in compliance with GLP also investigated Vantocil IB (20 % aqueous solution), batch AGDM 2253/77, in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 ± S9 mix from livers of Aroclor 1254 induced Sprague-Dawley rats. Test substance concentrations were up to 500 µg/plate. Vantocil IB gave negative response with all strains ± S9 mix. All tester strains were sensitive to the appropriate positive controls (N-2-fluorenylacetamide for TA98 and TA1538; 1,3-propanesultone for TA100 and TA 1535 and 9-aminoacridine for TA1537).

Ref. 92

SCCS comment

In an earlier bacterial reverse mutation test Vantocil P was toxic in several bacterial strains and showed to be mutagenic. As Vantocil P is a bactericide the SCCS considers the Ames test as not suitable for mutagenicity testing. This is also stated in a revised version of Notes of Guidance.

***In vitro* mammalian cell gene mutation test**

Guideline: OECD TG 476
 Species/strain: L5178Y TK+/- Mouse Lymphoma cells

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Replicates:	2
Test substance:	PHMB P20D
Vehicle:	culture medium
Batch:	1202
Purity:	/
Concentrations:	-S9 mix: 0, 12.5, 25, 50 and 100 µg/ml +S9 mix: 0, 12.5, 25, 50 and 100 µg/ml
Positive control:	-S9 mix: methyl methanesulfonate (MMS, 2 and 10 µg/ml)
Positive Control:	+S9-mix: cyclophosphamide (2 µg/ml)
Negative control:	solvent control
Treatment	3 h and 20 h
GLP:	yes
Study period:	2002

L5178Y TK+/- mouse lymphoma cells, suspended in treatment medium, were exposed to the designated concentrations of the test substance for 3 hr (in the presence or absence of a metabolising system (S9 from rat livers of Aroclor 1254 treated Sprague-Dawley rats, at 5 %) and for 20 hr (in the absence of S9). After washing, cells were plated for determination of viability and determination of survival and mutagenesis. Experiments with solvent control and positive controls were carried out under the same conditions.

Results: at 50 and 100 µg/ml, cytotoxicity of PHMB was higher than that of the positive controls. No significant increases in mutant frequencies were observed for PHMB in two independent assays at any concentration tested in the presence or absence of S9-mix. Analysis of size of colonies did not show any change in small and large colony number at any concentration tested in the presence or absence of S9-mix. MMS on the other hand produced clear increases in mutation frequencies in the absence of S9-mix and cyclophosphamide produced significant increases in mutation frequencies in the presence of S9-mix. Both positive controls produced a clear increase in small colony levels. Thus, positive and negative controls confirm the validity of the assay.

From the results of the study it can be concluded that PHMB is not mutagenic under the conditions of this assay.

Ref.: Harmand (2002)

SCCS comment

SCCS noticed that in all experiments performed, both short-term (with and without metabolic activation) and long-term treatment, at least one of the data points exhibited a statistically significant increase in mutant frequency compared to the concurrent negative control. Moreover, the increase in mutant frequency was concentration-related at least at one sampling time with metabolic activation.

However, in the recently adopted last version of the OECD TG on the gene mutation test at the *tk* locus, a positive result is determined with the global evaluation factor (GEF). If the test is performed according to the micro-well method an increase in the mutant frequency of 126×10^{-6} is considered a positive result. Since the increase in the present experiment was lower than this global evaluation factor, SCCS agrees with the conclusion of the authors that the test is negative.

Guideline:	similar to OECD TG 476
Species/strain:	Mouse lymphoma cell line P388 (<i>tk</i> +/-)
Replicates:	5 replicate cultures
Test substance:	Vantocil P (20% aqueous solution)
Vehicle:	DMSO
Batch:	ADGM/1021/79
Purity:	/
Concentrations:	-S9 mix: 25, 250, 500, 1000, 2000 µg/ml

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Positive control:	+S9 mix: 25, 250, 1000, 2000 µg/ml
Negative control:	±S9 mix: methyl methanesulfonate (MMS), 32.5 and 65 µg/ml
Treatment	DMSO
GLP:	yes
Study period:	1980 - 1981

PHMB was diluted with DMSO and added to the cell cultures for 30 minutes at indicated concentration levels and the cell suspensions in the test tubes were rotated on a flat bed test tube roller. S9 was obtained from livers of male Sprague-Dawley rats treated with Aroclor 1254. After exposure, the cells were washed and maintained in medium for an expression time between 68 – 72 hr at 37° C. Thereafter, 5-iodo-2'-deoxyuridine (IUDR) as selective agent and molten agar were added to the test tubes. The mixtures were shaken, dispensed into petri dishes and placed in a CO₂ incubator until colony formation. The colonies were counted and the mean of 5 replicates was determined. Positive and negative controls were tested in parallel.

The concentration of 2000 µg/ml was cytolethal and clear cytotoxicity was noted at 1000 µg/ml ± S9 mix. A sufficient number of surviving colonies for evaluation was observed at concentrations in the range between 25 – 1000 µg/ml ± S9 mix. No increase in mutation frequencies was observed at any PHMB concentration ± S9 mix. The positive control MMS induced a distinct increase in mutant colonies ± S9 mix indicating that the test was sensitive and valid.

The study authors concluded that PHMB was not mutagenic as it 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.

Ref.: 1

SCCS comment

Exposure time is lower than recommended in OECD TG 476. Additionally, only short time exposure was used and no longer exposure as recommended by the recently revised guideline. The cell line used does not belong to those recommended in OECD TG 476. Therefore, the results have limited value.

In vitro Micronucleus Test in human lymphocytes

Guideline:	OECD TG 473
Species/strain:	Cultured human peripheral blood lymphocytes from two volunteers (male and female)
Replicates:	Duplicate cultures, two independent experiments
Test substance:	Vantocil IB (19.6% aqueous PHMB solution)
Batch:	Bx2125
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:	0.85% physiological saline
Positive Controls:	-S9 mix: Mitomycin C (MMC), 0.5 µg/mL +S9 mix: Cyclophosphamide (CPA), 100 µg/mL
GLP:	Yes
Study period:	1989

The potential of PHMB to induce chromosome aberrations was investigated in cultured human peripheral blood lymphocytes from two volunteers in the presence or absence of S9 (from livers of male Alpk:APfSD rats induced with Aroclor 1254). PHMB was administered to

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the cultures approximately 44 hr after initiation and remained in contact with the cells for 2 hr 20 minutes to 3 hr 40 minutes. 70 hr after culture initiation, dividing lymphocytes were arrested in metaphase by treatment with 10 µg/ml colchicine and 2 hr later the cells were subjected to hypotonic treatment for 12 minutes in 0.075M KCl followed by fixation in glacial acetic acid/methanol and staining in Giemsa. The appropriate concentration levels selected for chromosomal analysis were 50, 25 and 5 µg/ml for both volunteers -S9 and 250, 100 and 25 µg/ml for volunteer 2 +S9 and 187.5, 100 and 25 µg/ml for volunteer 1 + S9.

Results:

The overall mitotic activity was reduced by approximately 50 - 80 %. In any case, PHMB could be examined for clastogenicity at concentration 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. The sensitivity and validity of the test system used was demonstrated as positive controls induced a significant increase in cells with structural aberrations.

The study authors concluded that PHMB did not induce chromosomal aberrations in cultured human lymphocytes under the test conditions applied.

Ref.: 54

SCCS comment

For donor 2, there was a depression in mitotic index recorded as well as a reduction in the overall number of cells present. However, the study authors considered that sufficient cells had been analysed for donor 2 not compromising the overall results. Only short time exposure time was used and not 24h as recommended by recent guideline.

In an earlier GLP-compliant study the potential of Vantocil P (20 % aqueous solution, Batch ADGM/1021/79) to induce chromosomal aberrations was investigated in cultured human lymphocytes from one donor (concentrations used: 1, 5, 10, 20 and 50 µg/ml Vantocil P, no information on treatment time) without external metabolising system. Mitomycin C was used as positive control, water (vehicle) was used as negative control. PHMB did not induce any concentration-related or statistically significant increase in chromosome damage up to concentrations, which led to decreases in the mitotic index.

Thus, the study authors concluded that Vantocil P did not exhibit a clastogenic potential in human lymphocytes exposed *in vitro* without a metabolic activation system.

Ref.: 84

SCCS comment

The experiment was not performed according to OECD test guideline. There is no information on treatment time. The study results have limited value and can be used only as supporting information.

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

In vivo Mammalian Bone Marrow Micronucleus Test

Guideline:	/
Species/strain:	Mouse, C57BL/6JfCD-1/Alpk
Group size:	5 males, 5 females per group
Test substance:	Vantocil IB (19.6 % aqueous PHMB solution)
Batch:	BX 2125 Ex. Grangemouth
Purity:	100 %
Dose levels:	0, 250, 400 mg/kg bw (no statement on correction for concentration)
Exposure:	Single application
Route:	Oral (gavage)

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Vehicle:	Double deionised water
Positive control:	Cyclophosphamide (CPA, 65 mg/kg bw)
GLP:	yes
Study period:	1988 - 1989

Dose levels were selected based on the findings of oral dosing of 500 and 2500 mg/kg bw followed by a 4-day observation period. Test substance was applied at dose levels of 0, 250 and 400 mg/kg bw. Animals were killed at 24, 48 and 72 hr after dosing (animals treated with positive control were killed at 24 hr only) and bone marrow was obtained, processed, and stained. 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.

Results:

No mortality occurred in any male or female mouse at any dose level. The high dose of 400 mg/kg bw/day led to a reduction in polychromatic erythrocytes as an indication of availability of substance in the bone marrow. No statistically significant increase in the frequency of Micronucleated polychromatic erythrocytes (MPE) was observed 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 hr sampling time. 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 hr 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 positive control caused chromosomal aberrations.

Based on these findings the study authors concluded that under the conditions applied, Vantocil IB is not clastogenic *in vivo*.

Ref.: 83

***In vivo* Unscheduled DNA synthesis**

Guideline:	mainly 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:	Vantocil IB, 19.6 % aqueous PHMB solution
Batch:	BX 2125
Purity:	/
Dose levels:	0, 750, 1500 mg/kg bw (no correction for concentration or purity)
Exposure:	Single application
Route:	Oral (gavage)
Vehicle:	for test substance: Double deionised water For positive control: corn oil
Positive control:	6-p-dimethylaminophenylazobenzthiazole (6-BT, 40 mg/kg bw)
Exposure period:	4, 12 h after application
GLP:	yes
Study period:	1989

Dose levels were selected based on ref. 55. Vantocil IB was administered by gavage at dose levels of 375 (not evaluated), 750 and 1500 mg/kg bw (application volume: 10 ml/kg bw) for a period of 4 or 12 hr to groups of 2 - 3 male rats per dose and experiment. Two independent experiments were carried out. The application volume was 10 ml/kg bw. After the respective treatment period, animals were sacrificed, liver perfusion was carried out and primary hepatocytes cultures were established from each animal. Hepatocyte cultures were

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exposed for 4 hr to [³H]-thymidine, slides were prepared and net nuclear grain counts were determined by counting 100 cells per animal.

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 Vantocil IB revealed no UDS induction in the hepatocytes of the treated animals as compared to the corresponding vehicle controls. The positive control produced the expected response.

The study authors concluded that Vantocil IB did not induce DNA repair as measured by unscheduled DNA synthesis in hepatocytes from rats exposed *in vivo*.

Ref.: 93

SCCS conclusion on Mutagenicity/Genotoxicity

The bacterial reverse mutation test is considered not suitable to be used as PHMB is a bactericide. PHMB was negative in a well performed 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.

Consequently, based on the available data PHMB can be considered to have no genotoxic potential and additional tests are unnecessary.

3.3.7 Carcinogenicity

3.3.7.1 Oral

Mouse

Guideline:	US EPA 83-2 (comparable to OECD TG 453)
Species/strain:	Mouse, C57Bl/10J/CD-1 Alpk
Group size:	55/sex/dose
Test substance:	Vantocil P (20.2% aqueous PHMB solution)
Batch:	D4097
Purity:	impurities < 0.76 %
Vehicle:	
Dose levels:	0, 400, 1200 and 4000 ppm (correction for 20.2 % PHMB content)
Route:	oral
Administration:	diet, 2 years
GLP:	yes
Study period:	1992 – 1994, report dated 1996

Dietary levels of PHMB corresponded to about 0, 55, 167, 715 mg/kg bw/day in males and 0, 69, 217, 856 mg/kg bw/day in females. The animals were examined for mortality and clinical signs including behaviour. Body weight and food consumption were determined at regular intervals. Blood samples for haematology 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 anaesthetised, 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.

Results

Non-neoplastic findings

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4000 ppm was considered as a dose exceeding the MTD. Body weights were reduced up to 20 % (males) and 15 % (females) compared to controls in the second year of the study. Compared to controls, the reduction of body weight gain was 35-42 % (males) and 22-33 % (females) during weeks 53- 79 of the study. Food consumption and mortalities were increased, food utilisation was decreased. Haemangiosarcoma was the most frequent factor causing death. 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, haematocrit and red cell count in both sexes receiving 4000 ppm. A variety of non-neoplastic changes was seen in the liver, gall bladder and recto-anal junction.

1200 ppm was considered as MTD. Compared to controls, body weights were 5 -6 % lower during the 2nd year of the study and the reduction of body weight gain was 7 - 14 % (males) and 5 - 10 % (females) during weeks 53- 79 of the study. There were non-neoplastic findings of inflammatory nature in the recto-anal junction and non-neoplastic changes in livers (both sexes) and gall bladder (females only). In females, haemoglobin, haematocrit and red cell count were increased. There was also an increase in the number of mice with torn ears and hairloss at 400 and 1200 ppm.

At 400 ppm, non-neoplastic findings consisted mainly in minimal inflammation of ano-rectal junctions.

Neoplastic findings:

Mice receiving 400 or 1200 ppm showed a reduced incidence of lymphosarcoma of the lymphoreticular system in comparison to controls.

At 4000 ppm there were increases in squamous cell carcinomas of the recto-anal junction in mice of both sexes (5 males and 8 females at 4000 ppm), one adenocarcinoma at the same site in a male from this group and a squamous cell carcinoma of the skin adjacent to the anus. Gall bladder papillomas occurred in two males at 4000 ppm. None was seen in controls or in mice from lower dose groups.

The highest incidence of treatment related tumours at 4000 ppm was in neoplasms of vascular origin, i.e. haemangiosarcomas, which are malignant tumours originating from vascular endothelium. As the altered tumor profile was present at a dose exceeding the MTD and as haemangiosarcoma is a common tumor in C57 mice, the findings of the study were reevaluated by a pathology working group. Although no statistical analyses were presented, the incidences of haemangiomas and of haemangiosarcomas (and combined incidences thereof) in the liver and any sites of high dose male and female mice were considered to be clearly above control incidences. In males at 1200 ppm, the increase of haemangiosarcomas in the liver was considered small.

Ref.: 74

The study was discussed by several expert groups.

As the altered tumor profile was present at a dose exceeding the MTD and as haemangiosarcoma is a common tumor in C57 mice, the findings of the study were first reevaluated by a pathology working group. Although no statistical analyses were presented, the incidences of haemangiomas and of haemangiosarcomas (and combined incidences thereof) in the liver and any sites of high dose male and female mice were considered to be clearly above control incidences. In males at 1200 ppm, the increase of haemangiosarcomas in the liver was considered a chance event. After statistical analysis of the same data 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. More recently, RAC stated that it would be unlikely to explain higher rates of vascular tumour in the liver by chance and that this study gives some evidence of carcinogenicity.

Ref.: 70, 37, 30

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There exists another, older non-GLP and non-guideline carcinogenicity study in mice with low reliability due to the high fighting-related mortalities during the first 6 months. Groups of 30 male and 60 female Swiss-derived albino mice were fed diets containing 0, 500, 1000 or 5000 ppm PHMB (Baqueacil SB (20 % aqueous PHMB solution), batches: SDC/596 and ADGM 5911), equivalent to 0, 100, 200 and 1000 ppm a.i. for one week prior to pairing and during mating. Feeding was 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.

Mortality was high due to high fighting of the males. Mean body weight gain in females was dose-dependently decreased and absolute liver weights in males and females were increased at 1000 ppm (significant for females only). There were no treatment-related (non-neoplastic or neoplastic) increases in histopathologic findings. However, with respect to vascular tumours of concern there were some treated animals bearing haemangiomas or haemangiosarcomas in the liver or at other sites.

Ref.: 18

Rat

A combined chronic toxicity/carcinogenicity study performed in rats is described in section 3.3.5.3.

There exists another, older non-GLP and non-guideline compliant long-term feeding study in rats which is of questionable reliability due to infections and less than 50 % survival at the end of study. Groups of 60 male and 60 female rats of an unspecified strain were fed PHMB (Baqueacil 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. There were 2 outbreaks of infection. The respiratory infection at week 70 and week 103 caused a rise in the mortality rates. No observable clinical effects were produced by the compound and apart from a slight anaemia in the top dose at 104 weeks, no adverse changes were seen. The administration of Baqueacil SB caused some growth depression in treated animals. Long-term exposure to PHMB was not related to toxic or carcinogenic effects. Haemangiomas were found at interim kills at week 52 in 1/12 male rats (mesenteric lymph nodes) at 200 ppm and 1/12 male rats at 200 ppm (cervical lymph nodes), at interim kill at 104 weeks in 2/12 males at 1000 ppm (mesenteric lymph nodes) and in 1/8 females at 200 ppm (uterus) as well as at the end of study at week 124 in 1/20 males at 1000 ppm (mesenteric lymph nodes), 1/19 males at 2000 ppm (spleen) and one haemangiosarcoma at week 104 in 1/21 males at 2000 ppm (mesenteric lymph nodes). No vascular tumours were seen in controls.

Ref.: 4

3.3.7.2 Dermal

Guideline:	Precedes guideline programs
Species/strain:	Mouse, Alpk:APfCD-1
Group size:	50/sex/dose
Test substance:	Baqueacil SB, (20.0 % aqueous PHMB solution)
Batch:	SDC/596, ADGM 5911
Purity:	
Vehicle:	ethanol
Dose levels:	0, 0.6, 6.0, 30 mg/mouse/day (corresponding to about 0, 15, 150, 750 mg/kg bw/day)
Dose volume :	
Route:	dermal, non-occluded

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Administration: daily, 80 weeks
 GLP: precedes GLP
 Study period: report dated 1977

Aqueous PHMB formulated in ethanol was applied to the shorn backs of groups of 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. Clinical observations (including ophthalmoscopy), bodyweights and food consumption were recorded. All animals were subjected to a post mortem examination. A full range of tissues were taken for histopathological examination.

Results:

The highest dose of 30 mg/mouse/day was considered of exceeding the MTD and led to irritant effects on the skin and to a general poor condition, which was accompanied by body weight loss and a high incidence in mortality (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. 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.

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. An overall NOAEL of 0.6 mg/mouse/day corresponding to 15 mg/kg bw/d was derived from this study.

Ref.: 17

SCCS conclusion on carcinogenicity

In a rat oral chronic toxicity/carcinogenicity assay, an increased incidence of haemangiosarcomas (statistically not significant) was observed at the highest dose of 2000 ppm PHMB. Recently it has been concluded (Ref. 30) 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 (750 mg/kg bw/d; considered to exceed MTD) tested. Based on this information and 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.

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

Guideline:	/
Species/strain:	Rat/Alpk:APfSD (Wistar derived)
Group size:	26/sex/dose
Test substance:	Vantocil P, 20.2% aqueous PHMB solution
Batch:	D4097
Purity:	0.76 % impurities
Dose levels:	0, 200, 600, 2000 ppm
Route:	Oral (diet)
Exposure period:	Two successive generations including 10 week premating period
GLP:	Yes
Study period:	1993/1994, report dated 1995

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Groups of animals received diets containing 0, 200, 600 and 2000 ppm Vantocil P (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, animals were mated and allowed to rear F1 litters to weaning. Selected F1 animals from F1 offspring were used as parental F1 animals for production of F2 offspring after an 11-week premating period. 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. At termination, gross pathology was performed, weights of kidney, liver, testes and epididymis were determined. Selected organs, grossly abnormal tissues and reproductive organs were processed 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 F1 pups/sex and 10 F2 pups/sex received a full post mortem examination.

Results:

In adults receiving the highest dose, there was a decrease in body weight and decreased efficiency of food utilisation during the pre-mating period, partly accompanied by reduced food consumption. There was no evidence of an effect of PHMB on reproductive parameters or on offspring growth and development up to the highest dose tested. There were some differences in the weights of livers and kidneys in adults receiving 2000 ppm Vantocil P and in F2 offspring at all dose levels. In the absence of related pathological changes these were considered not to be biologically significant. There was no evidence of an effect of Vantocil P on any of the reproductive parameters assessed. There was no evidence of an effect of PHMB on any of the pup parameters measured including growth and development. Based on decreased body weights in parental animals the authors derived a systemic, parental NOAEL of 600 ppm (70 – 79 mg/kg bw/d). The NOAEL for reproductive and offspring effects was 2000 ppm (239 – 270 mg/kg bw/d).

Ref.: 73

3.3.8.2 Other data on fertility and reproduction toxicity

Guideline:	US EPA 83-4 with some deviations
Species/strain:	Rat, Sprague-Dawley
Group size:	10 males, 20 females/dose
Test substance:	PHBM, 20 % aqueous solution
Batch:	ADGM 5642
Purity:	/
Dose levels:	0, 200, 650, 1300 ppm (dietary levels adjusted for 20 % a.i.)
Route:	oral, diet
Exposure period:	9 week premating period until 3 rd generation
GLP:	preceded GLP
Study period:	1975-1976

Ten to 12 week old animals were exposed for 9 weeks and then mated to produce F1 litters. From these litters 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 F3 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. 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 F3 pups were necropsied and representative tissues stored for possible future histopathological examination.

Results:

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

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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 (corresponding to approximately 130 mg/kg bw/d) was concluded to be the NOAEL for general toxicity, reproductive function and fertility as well as for pre- and postnatal development.

Ref.: 94

3.3.8.3 Developmental Toxicity

Rats

Guideline:	Comparable to OECD 414
Species/strain:	Rat/Alderly Park strain
Group size:	20 rats per group evaluated
Test substance:	Baquacil SB (20 % aqueous PHMB solution)
Batch:	no data
Dose levels:	0, 200, 1000, 2000 ppm (expressed as a.i.) (corresponding to about 0, 13, 54, 112 mg/kg bw/day)
Route:	Oral (diet)
Exposure period:	gestation days 1 – 20 (mating day taken as GD 0)
Positive control:	Aspirin, 2900 ppm
GLP:	/
Study period:	1976

Successfully mated female animals received diets containing test substance or aspirin from GD 1 until GD 20 when animals were killed. Body weight and food consumption were determined at regular intervals. Dams were examined for mortality, clinical signs and abortion. Fetuses were removed by Caesarian section, sexed, weighed and further investigated for external findings; uteri were examined for resorptions. About one half of the fetuses of each litter were examined for soft tissue findings and the remaining fetuses for skeletal findings.

Results:

There were no mortalities and no adverse clinical effects in any group. Maternal weight gain was significantly reduced in the groups receiving 1000 or 2000 ppm Baquacil SB and the food consumption was also significantly reduced in these groups. 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. No dose-related effects were observed on fetal or litter weights. There was an increase in extra ribs at the high dose which was considered to be a consequence of maternal toxicity at this dose level. There was no further substance-related effect on fetal morphology including ossification of the skeleton in any of the treated groups. Based on reduced food consumption and body weight, a maternal NOAEL of 200 ppm (corresponding to about 13 mg/kg bw/d) and a developmental NOAEL of 1000 ppm (corresponding to about 54 mg/kg bw/d) were derived.

Ref.: 46

Mice

Guideline:	Comparable to OECD 414
Species/strain:	Mouse/Alderly Park strain
Group size:	47 – 49 mice in dosed groups; 25 control animals
Test substance:	Baquacil SB (20 % aqueous PHMB solution)
Batch:	no data
Dose levels:	0, 10, 20, 40 mg/kg bw/d (expressed as a.i.)
Vehicle:	0.5 % aqueous Tween 80

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Route:	Oral (gavage), dose volume 10 ml/kg bw
Exposure period:	gestation days 6 – 15 (mating day taken as GD 0)
GLP:	/
Study period:	1976

Dose levels were based on a preliminary study. Successfully mated females received test substance or vehicle from GD 6 – 15. 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. 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. Twenty litters per group were examined in detail and the remaining fetuses preserved.

Results:

There were no mortalities and no substance-related adverse clinical signs; mean body weight was slightly reduced at 40 mg/kg bw/day. The mean pregnancy rate over all groups (including controls) was 59 %, whereas the expected value was about 80 %. Gestational parameters such as implantation sites, pre- and post implantation loss, litter size and weight, resorptions were not influenced by the test substance at any dose level. Fetal weight and sex distribution was not affected by treatment. There were indications of slight retardation of ossification from examination of forelimb and hindlimb digits and numbers of caudal vertebrae at 20 and 40 mg/kg bw/d. The incidence of wide fontanelles and poorly ossified frontal bones in the skull at these dose levels indicated also retarded ossification. The study authors concluded that based on the conditions of this study the maternal NOAEL was 40 mg/kg bw/d and the developmental NOAEL was 10 mg/kg/d. the latter NOAEL was confirmed in a reevaluation of the study.

Ref.: 45, 33, 34

Rabbits

Guideline:	Comparable to OECD 414
Species/strain:	Rabbit, White New Zealand
Group size:	20 females/dose
Test substance:	PHBM (20.2 % aqueous solution)
Batch:	D 4097
Purity:	impurities < 0.76 %
Dose levels:	0, 10, 20, 40 mg/kg bw/d
Vehicle:	deionised water
Route:	oral (gavage)
Exposure period:	gestation days 8 – 20
GLP:	yes
Study period:	1993

Dose levels were selected based on two previously performed range-finding studies. Test substance was administered to 20 time-mated rabbits/dose by gavage on GD 8 – 20. Stability of dose levels was checked analytically. The dams were examined for mortality, clinical signs and abortion. Body weight and food consumption was determined at regular intervals. On GD 30 the animals were killed and a macroscopic examination was performed. Intact gravid uteri were weighed and the number of corpora lutea, number and position of implantations, live fetuses, intra-uterine deaths and fetal weights were determined. Fetuses were sexed, weighed, examined for external abnormalities and cleft palate and visceral abnormalities. Heads were cut and brains examined; carcasses were further processed for investigation of skeletal abnormalities.

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Results:

In dams at the top dose, the following effects were observed: increased mortality (6 dams), reduced food intake (-23 % compared to controls between GD 8-14, p<0.01) and reduced bodyweight gain. In animals which died on study there were macroscopic changes in the stomach or caecum consistent with irritation and inflammation at site of contact. Signs of recovery were evident post-dosing when this group showed increased food consumption and final body weights similar to controls. In fetuses there was no effect of PHMB on the number, growth or survival in utero except a slight increase in pre-implantation loss observed at 40 mg/kg (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. But the difference in preimplantation loss at 40 mg/kg was not statistically significant and could not be related to PHMB as the dosing period began after implantation. The postimplantation loss at 20 mg/kg was not seen at the highest dose and in the absence of dose-response relationship, this effect was not attributed to treatment. There was no evidence for teratogenicity. The percentage of fetuses with unossified 5th sternebrae or with fused 4th and 5th sternebrae was increased at the top dose, but in the absence of changes in fetal development associated with PHMB, this finding was considered as unlikely to be substance-related. A maternal NOAEL of 20 mg/kg bw/d and a developmental NOAEL of 40 mg/kg bw/d were derived from this study.

Ref.: 9, 7, 8

In an older, non GLP- and non-Guideline compliant teratology study, test material IL 780, SDGM 5642 was investigated in groups of 14 – 15 mated female White New Zealand rabbits at dose levels of 0, 10, 40 and 160 mg/kg bw/d after oral (gavage) administration. 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

Conclusions on reproductive toxicity

In an oral chronic toxicity 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.

3.3.9 Toxicokinetics

Guideline/method:	/
Species/strain:	Rat/ Alpk:APfSD (Wistar derived)
Group size:	3 – 5 /sex/ group
Test substance (unlabelled):	PHMB (20 % aqueous solution), no batch stated
Test substance (labelled):	[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
Study period:	1994, report dated 1995

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Labelled and unlabelled test substances were fractionated into low, medium and high molecular weight fractions (MWtf) by centrifugation. The experiment was subdivided in a bioavailability experiment, a biliary excretion experiment and an excretion and tissue retention experiment. Radiolabelled PHMB was admixed to unlabelled PHMB.

Biliary excretion experiment: 3 male and female bile duct cannulated animals received a single oral dose of unfractionated PHMB at 20 mg /kg bw. Bile and urine were separately collected at 3 and 6 hr and bile, urine and feces at 12, 24, 36 and 48 hr after dosing. Excretion of radioactivity in urine, feces and bile were comparable between sexes. Most of the radioactivity was excreted via feces over 48 hr (96.8 % in males and 98.9 % in females). In the same period, less than 3 % of the administered radioactivity was excreted with the urine and less than 0.2 % of the administered radioactivity was excreted in bile. Total radioactivity eliminated in feces, urine and bile was 99.2 % in males and 102.2 % in females.

Bioavailability experiment: Three groups of four male rats received each a single oral dose of a low, medium or high MWtf of PHMB. The experiment was terminated 72 hr after dosing. 94.9, 101.4 and 96.0 % of the applied radioactivity from the low, mid and high MWtf, respectively, was eliminated via feces. 5.2, 0.2 and 0.2 % of the low, mid and high MWtf were excreted via urine. At the end, residual carcasses contained 0.15 – 0.54 % of the administered dose.

Excretion and tissue retention experiment: 5 male and 5 female animals received the low molecular weight fraction of PHMB. The experiment was terminated 72 hr after dosing. Of the administered dose, males excreted 7.8 % via urine and 94.1 % via feces and females excreted 2.6 % via urine and 93.5 % via feces. In tissues, highest amounts of radioactivity were found in the livers (0.18 % of dose in males and 0.19 % of dose in females) and kidneys (0.03 % of dose in males and 0.04 % of dose in females). Lower concentrations were found in all other tissues investigated. The residual carcasses contained 0.22 and 0.28 % of the dose.

Ref.: 68

SCCS comments

Although guideline adherence is not explicitly mentioned, the study is considered in line with OECD TG 417 (apart from the fact that only one dose level was used) and has been properly conducted.

Guideline/method:

Species/strain:	Rat/ Alpk:APfSD (Wistar derived)
Group size:	5 /sex/ group
Test substance (unlabelled):	PHMB (20 % aqueous solution), no batch stated
Test substance (labelled):	[¹⁴ C]-labelled PHMB, specific activity: 1.85 GBq/4mmol hexamethylene diamine
Dose levels:	200 and 2000 ppm
Vehicle:	sterilised, double deionised water
Route:	oral (diet)
Exposure:	Single application
Duration:	14 days
GLP:	yes
Study period:	1994, report dated 1995

Animals were fed diets containing either 200 ppm or 2000 ppm unlabelled PHMB for 14 days. At the end of this period two groups of five animals of each sex received a single oral dose of diet, incorporating [¹⁴C]-PHMB, as a 9 % suspension in the dose vehicle (4 mL/kg bw). The high dose corresponded to 0.8 mg [¹⁴C]-PHMB/kg bw (2 MBq/kg bw) and the low dose to 0.08 mg [¹⁴C]-PHMB/kg bw (0.2 MBq/kg bw). Thereafter, the animals were housed in metabolism cages and excreta (urine, feces) were collected for 72 hr following the radiolabelled dose.

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Results:

The 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. The major parts of radioactivity were excreted during the first 24 hr and excretion was virtually complete within 72 hr.

Less than 1 % of the administered radioactivity was found in the contents of the gastrointestinal tract at both dose levels (less than 1 %). Tissues of the gastro-intestinal tract contained 0.02 % - 0.1 % of the administered radioactivity.

Remaining male and female carcasses contained 1.3 % and 1.1 % of the applied radioactivity at the low dose and 0.2 % of the applied radioactivity for either sex at the high dose. The study authors concluded that at 200 ppm PHMB, 4.7 % and 3.9 % of the administered doses were bioavailable in males and females, whereas bioavailability percentages were 3.0 % and 2.6 % in high dose males and females.

Ref.: 67

In an older, non GLP-compliant study using male Alderley Park rats, the pattern of radioactivity excreted over a period of 240 hr after a single oral dose of 20 mg/kg bw/d of radiolabelled PHMB to 5 animals was as follows: 5.6 ± 0.35 % excreted in urine, 93.1 ± 1.58 % excreted via feces and 0.2 % exhaled. After feeding a diet containing 20 ppm radiolabelled PHMB, highest amounts of radioactivity were detected in adipose tissue, followed by kidneys and livers. No radioactivity was detected in the brain.

By applying simple chromatographic and spectroscopic methods the study authors demonstrated 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-aminoethyl)-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

In a screening study on the possible absorption of PHMB after oral administration, ten NMRI mice received a single oral application of 2.0 mL radiolabelled PHMB ($[^{14}\text{C}]$ -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 hr 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 an x-ray film. 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 absorption of PHMB took place in mice after single oral application.

Ref.: 62

SCCS conclusion on toxicokinetics

From a properly conducted oral (gavage) single dose toxicokinetic study using different molecular weight fractions of PHMB, the low molecular weight fraction might be considered as the most bioavailable fraction of which males excreted 7.8 % via urine and 94.1 % via feces and females excreted 2.6 % via urine and 93.5 % via feces. In tissues, highest amounts of radioactivity were found in the livers and kidneys. The residual carcasses contained 0.22 and 0.28 % of the dose. From this study, 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 %.

In a repeat-dose dietary study, the principle route of excretion was also feces. Urinary excretion amounted to 2.3 % and bioavailability amounted to 4.7 %. The major parts of radioactivity were excreted during the first 24 hr and excretion was virtually complete within 72 hr.

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The SCCS does not consider PHMB to belong to substances with low bioavailability as absorption percentages of total radioactivity up to 8.5 % were observed in oral studies. The SCCS notes, that absorption rates up to 8.5 % of the applied dose are quite high for a polymeric substance. However, as proper structural identification has neither been performed in the oral TK nor in the dermal absorption studies (only total radioactivity has been determined in these studies), surprisingly high absorption rates for a polymer could be due to substance degradation.

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity / photo-irritation and photosensitisation

The purpose of the study was to determine the safety of the test material following repeated applications to human panellists and exposure of the sites to natural Florida sunlight.

Guideline:	Adaptation of the repeat insult patch test (RIPT) procedure of Draize
Species:	Human
Group size:	26 volunteers (13 males, 13 females, aged <21 - >60 years)
Test substance:	Baquacil SB (20 % aqueous PHMB solution)
Batch:	ADGM 3429 (4/2/75)
Vehicle:	Distilled water; 0.01 % SLS
Route:	occlusive epicutaneous application on the upper arm
Induction:	three times/week 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 hr
Skin readings:	during induction: prior to 2 nd through 9 th application and on Monday following 9 th application 48, 96 hr after application after challenge: 48, 96 hr after application
GLP:	not stated
Study period:	1976

Volunteers received patch applications of 20 x 20 mm patches of 0.4 ml of an aqueous solution of Baquacil SB corresponding to 1 % a.i., which had been supplemented by SLS (final concentration of SLS: 0.01 %) in order to increase dermal penetration. Patches were applied to the upper arms of the panellists three times per week for 3 or 4 successive weeks and removed 24 hr after each application. Immediately after patch removal, test sites were exposed for one hr to mid-day sun rays (panellists of 3 week treatment started sun exposure in week 2 only). Reactions to all patches were scored before starting the second and the subsequent applications (until the 9th application).

In week 6 of the test, challenge applications of the test material were made on fresh and previously patched sites and reactions to challenge applications were scored 48 and 96 hr after treatment.

Results:

During the induction phase, erythema and oedema were observed at certain occasions in 5 panelists. After challenge exposure, no reactions were observed.

SCCS comment

The SCCS does not consider HRIPT studies for determining sensitisation potential to be ethical.

From the results it can be concluded that Baquacil SB had some irritating but no photosensitizing potential.

SCCS conclusion on phototoxicity

One non-standardised photo-sensitizing test is available. No studies on photomutagenicity or photoclastogenicity have been performed. Based on the available data, PHMB cannot be considered as a photosensitising or phototoxic substance.

3.3.11 Human data**3.3.11.1 HRIPT Tests**

Guideline:	Insult patch test according to standards set by Japanese patch test study group.
Species:	Human
Group size:	45 volunteers (17 males, 28 females)
Test substance:	Cosmocil CQ (20 % aqueous PHMB solution)
Batch:	0237
Vehicle:	Purified water
Concentrations:	0.3, 0.6 and 1.5 % a.i.
Route:	Topical application to the medial surface of the upper arm
Exposure duration:	24 h
GLP:	Yes
Study period:	2001

Finn-chamber plasters dosed with the indicated test concentrations were applied to the skin of the medial part of the arm. The plasters were removed after 24 hr and skin reactions were examined 30 min after exposure (24 hr-reading) and 24 hr thereafter (48 hr-reading). Skin reactions were scored according to the "Standards set by the Japanese Patch Test Study Group."

Results:

Plaster dermatitis was observed in all test groups, including vehicle controls. Only the highest test material concentration caused skin reactions at both time points, whereas lower concentrations and vehicle produced only reactions at the 24 hr time point. Skin irritation indices of 6.6, 5.5, 5.5 and 8.8 were obtained for concentrations of 0 (vehicle control), 0.3, 0.6 and 1.5 % a.i. The study authors considered the test material free of undesirable primary skin irritation property as skin irritation indices of test material solutions were almost the same as the irritation index of vehicle control.

Ref.: 91

SCCS comment

The test report was the English translation of a Japanese test report. The SCCS does not consider HRIPT studies for determining sensitisation potential to be ethical. As the contribution of plaster dermatitis cannot be clearly discerned from test-article induced dermatitis, no clear conclusions can be drawn from this study. It can be assumed that the test material is slightly irritating under the conditions of this test.

Guideline:	HRIFT according to the method of Shelanski, 1951, Shelanski and Shelanski, 1953 and Stotts, 1980 ^{*)}
Species:	Human
Group size:	191 of 209 volunteers completed
	49 in panel 1, 114 in panel 2, 28 in panel 3
Test substance:	Vantocil IB (20 % aqueous PHBM solution)
Batch:	Code Y 00156/001/005
Vehicle:	distilled water

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Concentrations:	for induction: 2 % and 4 % a.i. For challenge: 0.05, 0.1, 0.2, 0.5, 1 and 2 % a.i.
Route:	dermal (topical application to the dorsal surface of the upper arm)
Exposure duration:	24 hr
GLP:	not stated
Study period:	1981

For induction, 2 x 2 cm patches moistened with 0.5 ml aliquots of sample dilutions were applied to the dorsal surface of the upper arms for 24 hr. Induction patches were applied 3 times per week for a total of 10 applications. Challenge patches were applied on the sixth week of the test schedule (approximately 2 weeks after the last induction). Skin reactions were scored during the induction period prior to the next application and during the challenge period at 48 and 96 hr after application on a scale with grades 0 – 5 (no clearly visible reaction – strong reaction spreading well outside the test side, bullous reaction).

Panel 1:

Induction was performed with 2 % a.i. for the first 6 patches, concentration was increased to 4 % due to low level of irritation. Due to skin reaction symptoms in some of the panellists, levels were reduced again to 2 % afterwards. At challenge, 8 of 49 subjects (16 %) had skin reactions at 2 % a.i., 7 of 49 subjects (14 %) had skin reactions at 1 % and 0.5 % PHMB and 2 subjects (4 %) subjects showed weak reactions at 0.1% PHMB.

Panel 2:

Induction was performed in 114 subjects with 4 % a.i. 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 % a.i. 18 of 114 subjects (16 %) showed skin reactions at 0.5 % a.i. and 7 out of 114 subjects (6 %) showed reactions at 0.2 % a.i. No reactions were observed at 0.1 % and 0.05 % a.i. The intensity of the reactions was generally lower compared to those observed in panel 1. Two other panellists had reactions which appeared during the incubation (rest) period as a result of the 2 % induction but were negative to the four lower challenge concentrations – likely to be allergic to the 2 % concentration only. Ten other panellists gave indications of sensitisation (probably weak) during late induction to 2 % and gave no reactions at challenge to the four lower concentrations – probably allergic to the 2 % concentration only.

Panel 3:

Induction was performed in 28 subjects with 2 % a.i. for the first 4-5 induction patches, the remaining induction patches were performed with distilled water only due to the results obtained for panel 1. At challenge the subjects received concentrations of 0.05 %, 0.1 %, 0.2 % and 0.5 % a.i. One of 28 subjects (3.6 %) reacted to the highest challenge concentration of 0.5 % a.i., all other subjects gave negative results.

The study authors concluded that (a) Vantocil IB with a concentration of 2 % a.i. is not capable of causing primary skin irritation and (b) Vantocil IB with a concentration of 2 % a.i. is capable of causing skin sensitisation humans which can be elicited at concentrations starting from 0.2 % a.i.

^{*)}Shelanski, H.A. (1951): J.Soc. Chem. 2, 324-331

Shelanski, H.A. and Shelanski, M.V. (1053): Proc. Sci. Sect. Toilet Goods Assoc. 19, 46

Stotts, J. (1980): in "Current Concepts in Cutaneous Toxicity", p. 41.

Ref.: 88

SCCS comment

The SCCS does not consider HRIPT studies for determining sensitisation potential to be ethical.

PHMB can be considered as a skin sensitizer in humans in concentrations of at least 2 %.

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3.3.11.2 Clinical patch tests, case reports and medical surveillance data

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. 86

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.: 87

In agreement to the above studies, in an earlier study (apparently a meeting abstract which was not available to the SCCS), 2 out of 374 patients (0.5 %) reacted positively to PHMB patch tested at 2.5 % PHMB in water.

(McFadden, 1998, cited in Ref. 30 and 86).

In addition, there are sporadic case reports on sensitisation to PHMB, such as, for example, from wet wipes.

Ref.: SCCS Ref. VI

(ref: Leysen J, Goossens A, Lambert J, Aerts O (2014) Polyhexamethylene biguanide is a relevant skin sensitizer in wet wipes. Contact Dermatitis 70:323-325

Based on medical surveillance information obtained between 2004 and 2007 from employees working with PHMB no case of skin sensitization due to PHMB was reported. 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.

Ref.: 38

SCCS conclusions on human data

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 can be considered of low frequency (up to 0.5 % in dermatitis patients).

3.3.12 Special investigations

3.3.12.1 Mechanistic study in liver haemangiosarcoma induction (*in vitro* and *in vivo*)

In vitro:

Guideline/Method: /
 Test system: SVEC4-10 mouse endothelial +
 RAW 264.7 mouse macrophage lines
 Replicates: 2 - 3
 Test substance: PHMB (not further specified)
 Batch: /
 Concentrations: Cytotoxicity assay in SVEC4-10 cells: 0, 1, 2, 3, 4, 5 ppm
 Cytotoxicity assay in RAW 264.7: 0, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5 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
 Study period: 2008

RAW 264.7 mouse macrophages were co-cultured with SVEC-10 mouse liver endothelial cells in various experimental conditions: pre-activation of macrophages with PHMB or lipopolysaccharide (LPS) and/or co-culture in presence of PHMB. Endothelial cell proliferation was analyzed by the incorporation of BrdU. Production of reactive oxygen species in macrophages treated with PHMB was detected by measurement fluorescence intensity after addition of dihydrorhodamine and by evaluation of TNF α and IL-6 in cell culture medium as quantified by ELISA. PHMB had no direct effect on liver endothelial cell proliferation, did not activate macrophages and presence of PHMB did not potentiate cell proliferation induced by LPS-activated macrophages.

In vivo:

Guideline/method: /
 Species/strain: Mouse, C57Bl
 Group size: 5 males/ group
 Test substance: PHMB (not further specified)
 Batch: /
 Dose levels: 0, 100, 200, 400, 1200, 4000 ppm
 Route: oral (diet)
 Exposure period: 7, 14 or 28 days
 GLP: no
 Study period: 2008

Animals received PHMB containing diets as indicated. Immunohistochemical detection of bromodeoxyuridine (BrdU) in mouse liver was used to quantify cell proliferation in liver endothelial cells. Liver hepatotoxicity was assessed by measuring alanine aminotransferase and aspartate aminotransferase in plasma obtained at sacrifice. Plasma endotoxin levels were quantified using an endotoxin assay kit. Oxidative stress was measured by detection of 8-Hydroxydeoxyguanosine (OH8dG) in isolated DNA from livers. PHMB did not induce hepatotoxicity at any concentration or time point. At 4000 ppm body weight was transiently decreased and there was a thinning of the stomach wall but at 28 days of exposure, no effect on body weight or liver weight was observed at any dose. PHMB increased cell proliferation in a dose-related manner at 1200 and 4000 ppm. Cell proliferation was also increased at 1200 ppm PHMB following 14 days exposure. PHMB increased plasma

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endotoxin, a known activator of macrophages, at 1200 and 4000 ppm for 28 days and at 100 and 200 ppm for 14 days but not for longer duration.

In vitro and *in vivo* results point to an indirect effect of PHMB on liver endothelial cells. The increase in endothelial cell growth occurred with a threshold at 400 ppm and was dose-related. Endotoxin-mediated activation of macrophages might be involved, but other mechanisms cannot be excluded.

Ref.: 64

In vitro Mammalian cell transformation assay

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:	Vantocil IB (19.6% aqueous PHMB solution)
Batch:	ORG/42/78, ADGM 2253/77
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 +S9 mix, cell suspensions ±S9 mix
GLP:	Yes
Study period:	1978 – 1979, report dated 1980

Vantocil IB, dissolved in DMSO, 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 S9 from livers of Aroclor 1254 induced Sprague-Dawley rats. Negative controls consisted of the vehicle DMSO + S9 mix or of cell suspensions ±S9 mix. Benzidine and Acrylonitrile were used as positive controls in experiment I and II, respectively. Cell suspensions were incubated at room temperature for 3 hr after addition of metabolic activation system and test solutions were added and incubated at room temperature for 3 hr. After transfer to petri dishes, survival or transformation of cells was examined after an incubation period of either 6 – 9 or 14 – 21 days in a humidified atmosphere.

Results:

Cytotoxicity was observed in the survival and transformation assay at concentrations of 250 µg/mL and above. 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 transformed colonies demonstrating the sensitivity and suitability of the test system. The study authors concluded that Vantocil IB did not induce cell transformations in Baby hamster kidney fibroblasts (BHK21/C13) in the presence of S9-mix when tested up to cytotoxic concentrations.

Ref.: 92

SCCS Comment

Only experiments with S9-mix were performed. No conclusion can be drawn from this study.

3.3.13 Safety evaluation (including calculation of the MoS)

Absorption through the skin DAp (%)	= 7.65 %
Amount of cosmetic product applied daily A (g/d)	= 17.4 g/d
Concentration of ingredient in finished product C (%)	= 0.3%

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Typical body weight of human	= 60 kg
Systemic exposure dose (SED) =	
A (g/d) x 1000 mg/g x C (%) /100 x Dap (%) /100 /60	= 0.066555 mg/kg bw/d
No adverse observed effect level NOAEL (104 week oral rat study)	= 36 mg/kg bw/d
Corrected NOAEL based on 8.5 % oral absorption	= 3.1 mg/kg bw/d

$$\text{MOS} = \text{Corrected NOAEL}/\text{SED} = 46$$

3.3.14 Discussion

Physico-chemical properties

PHMB is a polymer which in neat form represents a solid/powder of > 94.2 % purity. It has a good water solubility of around 40 % and is usually marketed as an appr. 20 % aqueous solution. Average molecular weights in the range between 2670 and 4216 Da have been reported.

Molecular weight distribution of the polymer was not provided.

Function and uses

PHMB is supported under Directive 98/8/EC for uses as a disinfectant and it is used as a preservative and as an antimicrobial agent. As a preservative, PHMB is used in cosmetics, personal care products, fabric softeners, contact lens solutions, hand washes, and more.

In cosmetics, PHMB is used as a broad spectrum preservative. It is freely water soluble and therefore widely used in water-based products which are most susceptible to microorganism growth. 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*.

PHMB is also used to preserve wet wipes; to control odor in textiles; to prevent microbial contamination in wound irrigation and sterile dressings; to disinfect medical/dental utensil and trays, farm equipment, animal drinking water, and hard surfaces for food handling institutions and hospitals; and to deodorize vacuums and toilets. PHMB is used in antimicrobial hand washes and rubs and air filter treatments as an alternative to ozone. PHMB is also used as an active ingredient for recreational water treatment, as a chlorine-free polymeric sanitizer, which is effective against a wide variety of microorganisms.

Further reported uses of PHMB are purification of swimming pool water, beer glass sanitisation, solid surface disinfection in breweries and short-term preservation of hides and skins.

Toxicological Evaluation

Acute toxicity

From two acute oral toxicity studies performed with PHMB in rats, an LD₅₀ value of 1049 mg/kg bw was reported in one of the studies and LD₅₀ values of 549 mg/kg bw for males and 501 mg/kg bw for females were reported for the other study. Based on these values,

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PHMB can be considered of moderate acute oral toxicity; classification as Acute Tox 4 H302 (harmful if swallowed) is justified.

Two acute dermal toxicity tests have been performed. No deaths occurred up to dose levels of 5000 mg/kg bw/d. Thus it can be concluded that PHMB is not acutely toxic by the dermal pathway.

Two acute inhalation toxicity studies have been performed which were not available to the SCCS for evaluation. Recently, RAC has identified one of the studies leading to LC₅₀ values of 0.29 mg/l for males, 0.48 mg/l for females and 0.37 mg/l for males and females combined as key study thus warranting classification as Acute Tox 2 H330 (fatal if inhaled) and indicating acute inhalation toxicity. According to the rules on classification of mixtures (see ECHA Guidance on the application of CLP criteria (SCCS Ref. III)) and under the assumption, that all other ingredients present in the finished cosmetics products can be considered as being not acutely toxic, the acute toxicity estimate of a mixture containing 0.3 % PHBM would be 96.6, i.e. a classification as acute toxic for inhalation would not be warranted.

Irritation and corrosivity

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/m³.

Skin sensitisation

Based on various guinea pig maximization- and Buehler tests, PHMB can be considered as a moderate to strong sensitizer in animals. 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).

The SCCS notes that classification as Skin Sens 1B H317 according to CLP criteria is currently proposed.

Dermal absorption

Four studies on percutaneous penetration of PHMB were not in compliance with SCCS requirements, qualities of the studies were compromised by poor sample description and the studies have not been performed with representative cosmetic formulations containing 0.3 % PHMB. After the commenting period which followed the publication of SCCS/1535/14 on 18 June 2014, a draft of a new *in vitro* percutaneous absorption study was provided by the applicant.

The new dermal penetration study was performed according to OECD TG 428 and SCCS basic requirements for dermal penetration studies and was in line with GLP principles. Despite some processing errors and an outlier and despite the fact the study is still in the state of a draft report it is considered acceptable for determination of dermal absorption of PHMB.

The SCCS notes, that 20 tape strippings were used to remove stratum corneum.

The SCCS further notes that no information was given on the position of radiolabel within the molecule.

The SCCS also notes that the applicant intends to defend the safety of PHMB for all types of cosmetics products, whereas dermal penetration was only investigated in one type of

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formulation (oil/water emulsion.) Based on the variability observed in the study, the unusual high number of tape strippings used and the fact that dermal penetration was only investigated in one type of cosmetic formulation. According to the SCCS basic criteria for Skin Absorption studies, the mean + 2 SD will be taken for MOS calculation, i.e. $3.49 + 4.16\% = 7.65\%$ or $207.94 + 247.54 \text{ ng equiv./cm}^2 = 455.48 \text{ ng equiv./cm}^2$.

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 (rat, mouse, beagle dog).

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.

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 NOAELs of 36 and 45 mg/kg bw/d were derived for 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 hepatitis. Further, the incidence of haemangiosarcomas was increased at the highest dose tested.

Mutagenicity

The bacterial reverse mutation test is considered not suitable to be used as PHMB is a bactericide. PHMB was negative in a well performed 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.

Consequently, based on the available data PHMB can be considered to have no genotoxic potential and additional tests are unnecessary.

Carcinogenicity

In a rat oral chronic toxicity/carcinogenicity assay, an increased incidence of haemangiosarcomas (statistically not significant) 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 (750 mg/kg bw/d; considered to exceed MTD) tested. 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

From a properly conducted oral (gavage) single dose toxicokinetic study using different molecular weight fractions of PHMB, the low molecular weight fraction might be considered as the most bioavailable fraction of which males excreted 7.8 % via urine and 94.1 % via feces and females excreted 2.6 % via urine and 93.5 % via feces. In tissues, highest amounts of radioactivity were found in the livers and kidneys. The residual carcasses contained 0.22 and 0.28 % of the dose. From this study, 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).

In a repeated dose dietary study, the principle route of excretion was also feces. Urinary excretion amounted to 2.3 % and bioavailability amounted to 4.7 %. The major parts of radioactivity were excreted during the first 24 hr and excretion was virtually complete within 72 hr. The SCCS does not consider PHMB to belong to substances with low bioavailability as absorption percentages of total radioactivity up to 8.5 % were observed in oral studies. The SCCS notes, that absorption rates up to 8.5 % of the applied dose are quite high for a polymeric substance. However, as proper structural identification has neither been performed in the oral TK nor in the dermal absorption studies (only total radioactivity has been determined in these studies). High absorption rate for the polymer could be due to its degradation.

Phototoxicity

No studies on photomutagenicity or photoclastogenicity have been performed. PHMB cannot be considered as a photosensitizer.

Human data

See sensitisation.

4. CONCLUSION

1. Taking into account the scientific data provided, SCCS is requested to give its opinion on the safety of Polyaminopropyl Biguanide (PHMB) when used as a preservative in cosmetics products up to a maximum concentration of 0.3%.

On the basis of the data available, the SCCS concludes that Polyaminopropyl Biguanide (PHMB) is not safe for consumers when used as a preservative in all cosmetic products up to the maximum concentration of 0.3%.

The safe use could be based on a lower use concentration and/or restrictions with regard to cosmetic products' categories. Dermal absorption studies on additional representative cosmetic formulations are needed.

2. SCCS is requested to address any further scientific concerns on the ingredient Polyaminopropyl Biguanide (PHMB) in particular regarding its use in spray formulation.

On the basis of the data available, the SCCS concludes that Polyaminopropyl Biguanide (PHMB) is not safe for consumers when used as a preservative in cosmetic spray formulations up to a concentration of 0.3%.

PHMB is used in a variety of applications other than cosmetics. General exposure data from sources others than cosmetics should be submitted for the assessment of the aggregate exposure of PHMB.

5. MINORITY OPINION

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6. REFERENCES

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Abbreviations

- ECHA: European Chemicals Agency
RAC: ECHA's Committee for Risk Assessment