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SCCS OPINION ON Polyaminopropyl Biguanide (PHMB)- Submission III

Ulrike Bernauer, Laurent Bodin, Leonardo Celleno, Qasim Mohammad Chaudhry, Pieter-Jan Coenraads, Maria Dusinska, Jeanne Duus-Johansen, Janine Ezendam, Eric Gaffet, Lodovico Corrado Galli, et al.

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Scientific Committee on Consumer Safety
SCCS

OPINION ON

Polyaminopropyl Biguanide (PHMB)

- Submission III -

The SCCS adopted this Opinion by written procedure

On 23 December 2016

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 **About the Scientific Committees**

2 Two independent non-food Scientific Committees provide the Commission with the scientific
3 advice it needs when preparing policy and proposals relating to consumer safety, public health
4 and the environment. The Committees also draw the Commission's attention to the new or
5 emerging problems which may pose an actual or potential threat.

6 They are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee
7 on Health, Environmental and Emerging Risks (SCHEER) and are made up of independent
8 experts.

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

12 **SCCS**

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

18
19
20 **Scientific Committee members**

21 Ulrike Bernauer, Laurent Bodin, Leonardo Celleno, Qasim Chaudhry, Pieter Jan Coenraads,
22 Maria Dusinska, Jeanne Duus-Johansen, Janine Ezendam, Eric Gaffet, Corrado Lodovico Galli,
23 Berit Granum, Eirini Panteri, Vera Rogiers, Christophe Rousselle, Maciej Stepnik, Tamara
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40 The Opinions of the Scientific Committees present the views of the independent scientists who
41 are members of the committees. They do not necessarily reflect the views of the European
42 Commission. The opinions are published by the European Commission in their original language
43 only.

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

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 **ACKNOWLEDGMENTS**

2
3

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28

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31

32

33

34 Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on
35 Polyaminopropyl Biguanide (PHMB) - Submission III, SCCS/1581/16, preliminary version of 23
36 December 2016

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1. BACKGROUND

Poly(hexamethylene) biguanide hydrochloride (PHMB) (CAS 32289-58-0 / 27083-27-8 / 28757-47-3 / 133029-32-0) with INCI name Polyaminopropyl Biguanide, is currently listed in Annex V (entry 28) of the Regulation (EC) No. 1223/2009¹ (Cosmetics Regulation) as preservative to be used in all cosmetic products up to a maximum concentration of 0.3%.

Polyaminopropyl Biguanide (PHMB) is classified as CMR 2 (Carc. 2) 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. The classification applies from 1st January 2015 and according to Art. 15 (1) of the Cosmetics Regulation, PHMB is considered prohibited as cosmetic ingredient from 1st January 2015.

However, Art. 15 (1) of the Cosmetics Regulation 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'*.

The SCCS published an opinion on the safety of PHMB in June 2014 successively revised in July 2015 (SCCS/1535/14)⁴ in which they concluded 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.

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

In May 2016, Cosmetics Europe transmitted a new safety dossier on PHMB that addresses the major issues raised by the SCCS notably i) a lower maximum concentration of 0.1%, ii) new dermal absorption studies on representative formulations and iii) aggregate exposure data.

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 http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_157.pdf

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1 **2. Terms of reference**

2

3 (1) *In light of the new data provided, does the SCCS consider Polyaminopropyl Biguanide*
4 *(PHMB) safe when used as preservative in all cosmetic products up to a maximum*
5 *concentration of 0.1%?*

6 (2) *Alternatively, taking into account the EU market data available, does the SCCS consider*
7 *Polyaminopropyl Biguanide (PHMB) safe when used as preservative up to a maximum*
8 *concentration of 0.1% in all cosmetic products with the exclusion of those product categories*
9 *(body lotion, hand cream and oral care) in which this ingredient is seldom used?*

10 (3) *According to the new data provided, does the SCCS consider Polyaminopropyl Biguanide*
11 *(PHMB) safe for use in sprayable formulations up to a maximum concentration of 0.1%?*

12 (4) *Does the SCCS have any further scientific concerns with regard to the use of*
13 *Polyaminopropyl Biguanide (PHMB) in cosmetic products?*

14

1

2 **3. OPINION**

3

4 **3.1 Chemical and Physical Specifications**

5

6 **3.1.1 Chemical identity**

7

8 **3.1.1.1 Primary name and/or INCI name**

9

10 Chemical name: Polyhexamethylene biguanide hydrochloride (PHMB)*

11 INCI Name: Polyaminopropyl Biguanide

12

13 *Abbreviation PHMB has been used throughout this Opinion

14

15 **3.1.1.2 Chemical names**

16

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

18

19 Other chemical names:

20 Poly(hexamethylenebiguanide hydrochloride)

21 Poly(iminocarbonimidoyliminocarbonimidoylimino-1,6-hexanediyl), hydrochloride

22 Poly(iminoimidocarbonyl-iminoimidocarbonyl-iminohexamethylene), hydrochloride

23 Poly(iminoimidocarbonyliminoimidocarbonyliminohexamethylene) hydrochloride

24

25 **3.1.1.3 Trade names and abbreviations**

26

27 Baquacil

28 Caswell No. 676

29 Cosmocil CQ

30 EPA Pesticide Chemical Code 111801

31 PHMB

32 Polihexanide

33 Polihexanido

34 Polihexanidum

35 Polyhexanide

36 PP 073

37 UNII-322U039GMF

38 Vantocil IB

39 Vantocil TG

40

Reference: Submission file, therein cited: TOXNET 2013

41

42 **3.1.1.4 CAS / EC number**

43

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

45

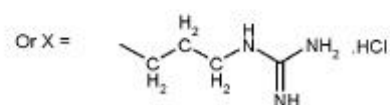
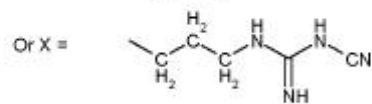
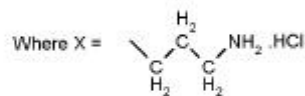
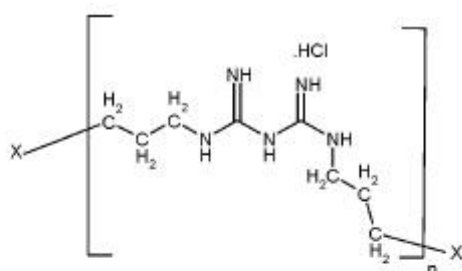
Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 Two equivalent CAS numbers can be allocated depending on how the polymer is described.
2 CAS-No 27083-27-8 expresses the PHMB in terms of its starting monomers (N,N''-1,6-
3 hexanediylbis(N'-cyanoguanidine) and 1,6-hexanediamine).
4 CAS-No 32289-58-0 expresses the PHMB as the resultant polymer.

5
6 EC: 608-723-9 and 608-042-7

7
8 Ref.: 124

9
10 **3.1.1.5 Structural formula**



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

13
14 Ref.: 2

15
16 **3.1.1.6 Empirical formula**

17
18 (C₈H₁₇N₅)_n.nHCl, n=1-40

19 Ref.: 2

20 **3.1.2 Physical form**

21
22 Off-white to pale yellow powder with strong ammonia smell at 20° C and 101.3 kPa

23 Ref.: 2; 30

24 Very pale yellow to pale yellow, lumpy solid; no obvious odour.

25 Ref.: 5

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

27 Ref.: 22

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

3.1.3 Molecular weight

Molecular weight:

> 700 g/mol (submission)

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

3686 – 4216 (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.

Submission III

Composition of a commercially used mixture:

- Molecular weight fraction < 500 dalton: 6% - made out of two monomers: Hexamethylenediamine (HMD): CAS 124-09-4, and Hexamethylenebis(dicyandiamide) (HMBDA): EC 240-032-4, CAS 15894-70-9
- Molecular weight fraction 500 to 1000 dalton: 14.1%
- Molecular weight fraction > 1000 dalton: 75.8%

Ref.:35, 121

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	Vantocil P, (Ref. 35) Samples: DR4529; DR4532; DR4535; DR4538; DR4541	Vantocil P, (Ref. 36) Samples: DR4529	PHBM, DS 6274 (Ref.: 23-26)	Five different batches of technical grade PHMB (Solid PHMB) (Ref. 22)
--------------------	---	---	---	-----------------------------	---

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

	10320310 ASG 10320311				
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-guanidinohexyl)guanidine (Ref. 35); according to Ref. 36, impurity A can be accounted for in part by HMBDA 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-aminohexyl)guanidine).

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

Impurity C: possibly N-cyano-N'-(6-guanidinohexyl)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, 2

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

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1 Acetone: 2.7 ppm at 22°C
2 Dichloromethane: 0.2 ppm at 22°C
3 Ethyl Acetate: 0.1 ppm at 22°C
4 Toluene: 0.2 ppm at 22°C
5 n-hexane: 0.1 ppm at 22°C
6 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 x 10⁻⁷ Pa (20°C) and 4.11 x 10⁻⁷ Pa (25°C)
(estimated according to OECD TG 104) (Ref. 16)
b) 6.0 x 10⁻⁸ Pa (20°C) and 2.0 x 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 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.

3.1.9 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.

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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.

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 those in the 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

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1 washes and rubs and air filter treatments as an alternative to ozone. PHMB is also used as an
2 active ingredient for recreational water treatment, as a chlorine-free polymeric sanitiser, which
3 is effective against a wide variety of microorganisms.

4
5 Further reported uses of PHMB are purification of swimming pool water, beer glass sanitisation,
6 solid surface disinfection in breweries and short-term preservation of hides and skins.

7
8 Ref.: submission dossier; 63, 28
9

10 **SCCS comments**

11 Recently PHMB has been assessed for biocidal uses by ECHA. The use of PHMB as a biocide has
12 been approved in product types TP 2,3,4,11 for which exposure to the consumers is limited
13 but not in product types TP1, 5, 6, 9 for which exposure may be significant. Therefore the
14 exposure of consumer to PHMB as a biocide will be significantly reduced due to these new
15 foreseen regulations.
16

17 **3.3 Toxicological Evaluation**

18
19 **3.3.1 Acute toxicity**

20
21 Submission II

22
23 **3.3.1.1 Acute oral toxicity**

24
25
26 Guideline: OECD TG 425
27 Species/strain: rat, Sprague-Dawley
28 Group size: /
29 Test substance: PHMB DS6274
30 Batch: PC 100-02
31 Purity: certificate of analysis was not attached; submission states 96.0 %
32 Vehicle: distilled water
33 Dose levels: 550 and 2000 mg/kg bw
34 Dose volume: 20 ml/kg bw
35 Administration: gavage
36 GLP: yes
37 Study period: 2002

38
39 A total of 6 female animals were dosed individually in sequence with at least 48 hours between
40 the animals at dose levels of 2000 or 550 mg/kg bw. All surviving animals were observed for
41 14 days post-dose.

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

44 Hunched posture and pilo-erection were noted in 2 animals treated with 2000 mg/kg just after
45 dosing. Signs of systemic toxicity also noted 1 day after dosing in 1 animal treated with 2000
46 mg/kg were lethargy, ataxia, decreased respiratory rate, laboured respiration, ptosis and tiptoe
47 gait.
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1 There were no signs of systemic toxicity noted in animals treated with 550 mg/kg. The
2 surviving animals showed expected gains in bodyweight over the study period. Abnormalities
3 noted at necropsy of animals that died during the study were haemorrhagic or abnormally red
4 lung, dark liver, dark kidneys, haemorrhage or sloughing of the gastric mucosa, sloughing of
5 the non-glandular epithelium of the stomach and haemorrhage of the small intestine. No
6 abnormalities were noted at necropsy in animals that survived through the 14-day observation
7 period.

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

9 Ref.: 23

10
11
12
13
14 **Guideline:** Considered equivalent to OECD 401 but without gross necropsy
15
16 **Species/strain:** Rat, Alderley Park
17
18 **Group size:** 5 / sex/ dose
19 **Test substance:** Vantocil P (20 % aqueous solution of PHMB)
20 **Batch:** BX 791/2[ADGM 1021/79]
21
22 **Purity:** no information given
23
24 **Vehicle:** deionised water
25
26 **Dose levels:** 700, 1000, 1500, 2000, 2500, 3000, 3500 and 5000 mg/kg bw
27 **Administration:** stomach tube
28
29 **GLP:** yes
30 **Study period:** 1979

31
32 Animals were fasted for 16 – 20 hrs and then given various doses of an aqueous solution of
33 PHMB by stomach tube at a standard volume of 10 ml/kg. Animals were observed for 14 days.
34 No deaths occurred in animals dosed at 700, 1000 or 1500 mg/kg bw. Signs of toxicity could
35 be observed in all dose groups and consisted of salivation, lacrimation, piloerection and in
36 subdued appearance for some animals. At 1500 mg/kg bw, further signs of toxicity were
37 wheezing and staining around the mouth. Toxic signs did not persist beyond day 7 or 8 of the
38 study. Cumulative mortalities in male rats were 1 at 2000 mg/kg bw, 2 at 2500 mg/kg bw, and
39 4 at 3000, 3500 and 5000 mg/kg bw, respectively. In female rats, cumulative mortalities were
40 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
41 5 at 5000 mg/kg bw. LD50 values of 2747 mg/kg and 2504 mg/kg were derived for male and
42 female rats, respectively corresponding to approximately 549 and 501 mg/kg bw a.i. in male
43 and female animals, respectively.

44
45 Ref.: 55

46 **SCCS comment**

47 According to Annex VI of Regulation (EC) No. 1272/2008 a harmonised classification as Acute
48 Tox 4 H302 (harmful if swallowed) has been assigned.

49
50
51 **3.3.1.2 Acute dermal toxicity**

52 **Guideline:** OECD TG 402 (EU B.3)
53 **Species/strain:** Rat, Sprague-Dawley

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1 Group size: 5 males and 5 females
2
3 Test substance: PHMB DS6274
4 Batch: PC 100-02
5
6 Purity: certificate of analysis was not attached; submission states 96.0 %
7
8 Vehicle: distilled water
9 Dose levels: 5000 mg/kg bw
10 Administration: dermal, semi-occlusive
11
12 GLP: yes
13
14 Study period: 2002
15

16 Clipped skin (10 % of the total body surface area of the dorsal and dorso-lateral parts of the
17 trunk) of animals was treated with a single dose of 5000 mg/kg bw of PHMB (moistened with
18 distilled water). The application area was covered by semi-occlusive dressing for 24 hours.
19 Body weights were determined shortly before administration and on days 7 and 14. Clinical
20 signs were recorded several times on the day of administration and at least once daily
21 thereafter. The skin was examined and the findings were scored according to Draize 0.5, 1, 2
22 and 4 hr after removal of the semi-occlusive dressing and subsequently once per week for 14
23 days. Animals were sacrificed after the observation period and gross pathological examinations
24 were performed.

25
26 No mortality occurred, no signs of systemic toxicity were observed and body weight gain was
27 not affected. Very slight to well-defined erythema was noted at the treatment sites of all
28 animals one and two days after dosing. Very slight erythema persisted at the treatment sites
29 of two animals three days after dosing. Hemorrhage of dermal capillaries was noted at the
30 treatment sites of eight animals one and two days after dosing. Small superficial scattered
31 scabs were noted at the treatment sites of all animals. No abnormalities were noted at
32 necropsy.

33 An acute dermal toxicity > 5000 mg/kg bw was derived from this study.
34
35
36

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
37 Administration: dermal, occlusive
38
39 GLP: yes
40
41 Study period: 1979
42
43
44
45
46

Ref. 55

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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, rhonchus, 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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1 Table 2: mortality of Wistar rats after inhalation of 0.1, 0.3 and 0.5 mg/l PHMB for 4 hr.
2

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.

3
4
5 Clinical signs:

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

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

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

36 Effect on body weight:

38 At 0.1 mg/L, approximately 8-11% body weight loss was observed in all males following the
39 exposure on Day 1. The body weight returned to the initial values mostly on Day 7, in 2 of 5

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1 animals between Days 7-14. In females slight body weight loss (about 4-6%) was recorded for
2 3 of 5 animals on Day 1. The body weight returned to the initial values approximately on Day 3
3 (in one female on Day 7).

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

9
10 **Necropsy:**

11 Enlargement of dark/red discoloured lungs and/or dark/red discoloration of the fur at the
12 perinasal and/or white foamy material in the trachea were seen in all animals found dead,
13 which were those animals exposed at 0.3 and 0.5 mg/L of PHMB, respectively. No test item
14 related macroscopic observations were noted in animals exposed to concentrations up to 0.5
15 mg/L at terminal sacrifice (Day 14).

16
17 On the basis of this study, LC₅₀ were determined to be 0.29 mg/l for males, 0.48 mg/l for
18 females and 0.37 mg/l for males and females combined (1.85 mg/L for 20% PHMB solution).”
19
20

21
22 Ref.:5, 30
23

24 **SCCS comment**

25 According to the harmonised classification and labelling (ATP09) approved by the European
26 Union, PHMB is fatal if inhaled (H330).
27

28 Ref.: SCCS Ref. V
29

30 According to the rules on classification of mixtures (see ECHA Guidance on the application of
31 CLP criteria (SCCS Ref. III)) and under the assumption that all other ingredients present in the
32 finished cosmetics products can be considered as being not acutely toxic, classification for a
33 mixture containing 0.1 % PHMB would not be warranted.
34

35 At a concentration of 0.1 mg/l no mortalities of Wistar rats are observed after inhalation for 4
36 hours.
37
38

39 **3.3.2 Irritation and corrosivity**

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41
42 **3.3.2.1 Skin irritation**

43
44
45 Guideline: OECD TG 404; EU B.4
46 Species/strain: Rabbit / White New Zealand
47 Group size: 3 male animals
48 Test substance: PHMB, DS6274
49 Batch: PC 100-02
50 Purity: reference is given to a non-attached appendix; submission states: 96 %

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 Vehicle: distilled water (to moisten neat substance powder)
2 Dose level: 0.5 g neat substance
3 Dose volume: 0.5 g neat substance moistened with 0.5 ml water
4
5 Observation: 7 days
6 GLP: yes
7
8 Study period: 2002, report dated 2003
9 One animal was initially treated with the moistened test substance on three sites of the back;
10 treated sites were then covered with a cotton gauze patch held in place by adhesive tape.
11 Patches were successively removed after 3 minutes, 1 hr and 4 hr. After inspection of skin
12 reactions in this animal, two further animals were treated with 0.5 g of moistened test
13 substance which was held in place for 4 hr. After treatment periods, patches and residual
14 materials were removed. Test sites were examined at the following time points after patch
15 removal: 1, 24, 48 and 72 hr for evidence of primary irritation and scored according the Draize
16 scheme.

17
18 **Results**

20 There was no evidence of skin irritation following exposures of 3 minutes or 1 hr. After the 4-hr
21 exposure the primary irritation index was 1.0. The mean value of 24, 48 and 72 hr for either
22 erythema and eschar formation or oedema formation calculated for each animal tested was 1
23 or less. Following the 4-hr exposure, well-defined erythema was noted at one treated skin site
24 with very slight erythema at 2 treated skin sites 1 and 24 hr after patch removal. Very slight
25 erythema was noted at all treated skin sites at the 48-hr observation and persisted at 1 treated
26 skin site at the 72-hr observation.
27 Slight oedema was noted at 1 treated skin site 1 hr after patch removal with very slight
28 oedema at the 24- and 48-hr observation. There was no skin reaction after 7 days.

30 **Conclusion**

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

33 Ref.:25; SCCS Ref. II

35 **Guideline:** preceded OECD test guideline
37 **Species/strain:** Rabbit, White New Zealand
38 **Group size:** 3 males, 6 females
40 **Test substance:** Vantocil P (20 % aqueous solution of PHMB)
41 **Batch:** BX 791/2[ADGM 1021/79]
43 **Purity:** no information
44 **Vehicle:** /
46 **Dose level:**
48 **Dose volume:** 0.5 ml
49 **Observation:** 72 hrs / 21 day
51 **GLP:**
53 **Study period:** 1980
54

55 Test substance was applied to an area of approximately 6.25 cm² intact and abraded skin of
56 the flanks of 6 female rabbits and kept for 24 hr under occlusive dressing. After patch removal,
57 the skin was examined at 24 and 72 hr for erythema and oedema and the skin irritation was

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 assessed using the Draize method. In a separate section of the study, three male animals
2 received the same treatment but were killed 48 (1 animal) and 72 hr (2 animals) after
3 substance administration and skin samples were taken for histopathological examination.

4
5
6 **Results**

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

12 Male animals: histopathological examination of intact and abraded skin sites indicated
13 moderate to marked acute inflammation characterised by epidermal acanthosis and a
14 polymorphonuclear cell infiltrate in the superficial corium. There were also areas of focal
15 necrosis extending slightly into the corium.

16
17
18
19 **Conclusion**

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

22
23
24 Ref.: 57

25
26
27 In a barely readable test report on a skin corrosivity test performed with Vantocil P (Batch BX
28 791/2[ADGM 1021/79]; 20 % aqueous formulation) in White New Zealand rabbits it was stated
29 that application of the material to the intact and abraded skin of six animals led to superficial
30 scabbing and erythema around the abrasions but there were no signs of necrosis to the intact
31 skin of any of the animals. Therefore it was concluded that a 20 % solution of Vantocil P is not
32 corrosive to the rabbit skin

33
34 Ref.: 56

35
36 In references not available to the SCCS, the application of solid PHMB (96 %) to the skin of
37 rabbits according to OECD 404 induced no signs of skin irritation. After dermal application of a
38 20 % aqueous PHMB solution moderate erythema was recorded 24 hr after application on the
39 treated area of all three animals. The reaction was completely reversible between days 6 and 8.
40 No oedema was observed with the 20 % aqueous solution.

41
42 Ref.: 30

43
44
45 **SCCS comment on skin irritation**

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

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52 **3.3.2.2 Mucous membrane irritation / Eye irritation**

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55 Guideline: OECD TG 405 (EU B.5)

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

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4 Species/strain: Rabbit, White New Zealand
5 Group size: 1 male animal
6 Test substance: PHMB DS6274
7 Batch: PC 100-02
8 Purity: reference is given to a non-attached appendix; submission states: 96 %
9 Vehicle: /
10 Dose levels: 0.1 ml neat substance
11 Administration: instillation in the conjunctival sac of the right eye
12 GLP: yes
13 Study period: 2002, test report 2003
14
15
16
17
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19

20 Neat test substance was instilled into the conjunctival sac of one eye of a male rabbit, the
21 untreated eye served as control. The eyes were not rinsed. Both eyes were examined at 1, 24,
22 48 and 72 hr as well as on days 7, 14 and 21 after instillation. The effects on the cornea, iris
23 and conjunctivae were scored according to the OECD guideline method. Due to the severity of
24 the ocular response, no additional animal was treated for animal welfare reasons.

25
26
27
28 Results

29 The single application to the non-rinsed eye of the animal led to opalescent corneal opacity,
30 iridial inflammation and severe conjunctival irritation. Other ocular effects were noted in the
31 form of dulling of the normal corneal lustre, vascularisation and pale appearance of the
32 nictitating membrane. Translucent corneal opacity, minimal conjunctival irritation and
33 vascularisation were noted at the 21-day observation and were considered as irreversible.
34

35 Conclusion

36 Under the conditions of the study, neat PHMB induced irreversible ocular damage and was
37 considered as corrosive to the rabbit eye.
38
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Ref.:26

44 Guideline: /
45 Species/strain: Rabbit, White New Zealand
46 Group size: 9 females
47 Test substance: Vantocil IB (20 % aqueous solution of PHMB)
48 Batch: ADGM 1021/79
49 Purity: no information
50 Vehicle: /
51 Dose volume: 0.1 ml
52 Observation: up to 35 days after instillation
53 GLP: yes
54 Study period: 1981
55
56
57
58
59
60

61 Test substance as supplied was instilled in the conjunctival sac of one eye; the other eye
62 remained untreated and served as control. The eyes of six animals were not irrigated after
63 instillation. The eyes of the other three animals were irrigated for one minute with lukewarm
64 water about 20 – 30 seconds after the instillation. The animals were examined and the grade

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1 of ocular reaction was recorded at 1 – 2 hr and 1, 2, 3, 4, 7, 8, 15, 25, 26, 28 and 35 days
2 after instillation according to the method of Draize (1959). Most of the animals showed signs
3 of slight to moderate initial pain following instillation. The animals whose eyes were not rinsed
4 showed iritis and conjunctivitis and 4/6 animals showed corneal opacity but recovered by day
5 25. The animals with the rinsed eyes showed conjunctivitis and one had slight iritis, but none
6 of them showed any corneal reaction. From the results of the study, the authors concluded
7 that Vantocil IB is a moderate eye irritant without irrigation and a mild irritant with irrigation.

8
9 Ref.: 75

10
11
12 In a recent study not available to the SCCS and performed according to OECD, TG 405 PHMB
13 supplied as powder (purity 99.6%) was instilled into the eye of one New Zealand rabbit at the
14 dose of 0.1 g. At the conjunctival level, a moderate redness was noted 1 hr after instillation
15 and still noted at the end of the observation at day 7. It was associated with an important
16 chemosis noted 24 hr after instillation and still noted at the end of the observation. At the
17 corneal level, a moderate opacity was registered 1 hr after instillation and still present at the
18 end of the observation. At the iris level, congestion was registered from the 2nd day of the
19 test and persisted until the end of the observation.

20
21 An ulceration of the nictating membrane and the cornea was noted from the 1st day of the
22 test. This lesion persisted for at least 72 h. Taking into account the severity of the reactions,
23 the study was stopped at day 7 in accordance with the principles of animal welfare and
24 additional animals were not treated.

25 Ref.:30

26
27
28 **SCCS comment**

29 According to Annex VI of Regulation (EC) No. 1272/2008, a harmonised classification as Eye
30 Dam 1 H318 (causes serious eye damage) has been assigned based on the above studies.

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32
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34 **3.3.3 Skin sensitisation**

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37 Guinea pig maximisation test

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48
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.)

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1 Positive control: 3 % [w/v] 2-mercaptobenzothiazole
2
3 GLP: yes
4 Study period: 1992, report dated 1993
5

6 Based on the results of range-finding studies, animals were intradermally injected with 0.1 ml
7 of a 0.06 % a.i. aqueous solution with or without Freund's complete adjuvant into the shaven
8 shoulder region of test group animals. One week later, dermal induction was performed by
9 occlusively applying neat substance (20.2 % a.i.) in the induction sites for 48 hr. Challenge was
10 performed by occlusive epicutaneous application of the undiluted material (20.2 % a.i.) and a
11 30 % solution in deionized water (6 % a.i.) to a previously untreated site for 24 hr. The
12 application sites were scored 24 and 48 hr after removal of the patch according to the scheme.

13 **Results:**

14
15 Following challenge with 20.2 % a.i., scattered mild redness or moderate diffuse redness was
16 observed in 18/20 of test animals at 24 hr and 16/20 test animals at 48 hr (average scores of
17 1.4 at 24 hr and 1.2 at 48 hr). Scattered mild redness was observed in 4/10 of the control
18 animals at 24h and 2/10 at 48h. The net frequency of response at 24 hr was 50 %.

19
20 Following challenge with 6 % a.i., scattered mild redness or moderate diffuse redness was
21 observed in 5/20 test animals at 24 hr and 2/20 at 48 hr (average scores of 0.3 at 24 hr and
22 0.1 at 48 hr) and scattered mild redness was observed only in 1 of the ten control animals at
23 24 hr. A strong sensitisation response was observed for the positive control.
24

25 The study authors concluded that PHMB should be considered as a moderate sensitiser.
26

27 Ref.: 27
28

29 **SCCS Comment**

30
31 Under the conditions of challenge with 20.2 % a.i. (50 % net frequency of response at 24 hr)
32 the substance should be considered as a strong sensitiser according to Regulation (EC) No
33 1272/2008 (CLP regulation).
34
35
36
37

38 A further guinea pig maximisation test performed according to OECD TG 406 and GLP principles
39 was not available for evaluation to the SCCS. The study has been evaluated by RAC and
40 reported in Ref. 30, from where the study description is cited:
41

42 "Another Guinea Pig Maximisation Test was performed according to guideline OECD 406 and
43 GLP (Richeux, 2002c) on 20% aqueous PHMB diluted in physiological saline. Intradermal
44 induction was performed with 0.15% PHMB and topical induction with 20% PHMB. Challenge
45 was performed with 20% or 10% PHMB. 24 hours after challenge moderate erythema was
46 observed in one animal out of 10 at the 20% challenge treatment site (net frequency of
47 response of 10%) and for one animal out of 10 at the 10% concentration site in the test group
48 (net frequency of response of 10%). No reactions were evident in the control group. 48 hours
49 after challenge, moderate erythema was observed in one animal at the 10% treatment site in
50 the test group (net frequency of response of 10%). No reactions were evident in the control
51 group. Under the conditions of this study, PHMB is not considered as a dermal sensitiser
52 according to classification criteria."
53

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 Two older guinea pig maximisation tests did not strictly adhere to OECD TG 406:
2
3 Guideline: considered comparable to OECD TG 406
4
5
6 Species/strain: Guinea pigs, Alderley Park
7
8 Group size: 20 test animals, 8 control animals, females
9
10 Test substance: Vantocil IB (20.2 % aqueous solution of PHMB)
11
12 Batch: [BX 791/2 (ADGM 1021)]
13
14 Purity: no information
15
16 Vehicle: deionised water
17
18 Concentration: intradermal induction: 1% of material as delivered (0.2 % a.i.)
19 dermal induction: material as delivered (20.2 % a.i.)
20 challenge: material as delivered (20.2 % a.i.)
21
22 Positive control: /
23
24 GLP: yes
25
26 Study period: report dated 1980

26 Animals received intradermal inductions to the clipped scapular region with 0.2 % PHMB in
27 deionised water and Freund's complete adjuvant (FCA), 0.2 % PHMB in water and FCA plus
28 water. 7 days later, animals received topical induction with a neat preparation of the test
29 article (20.2 % PHMB) to the same area which was held in place for 48 hr. Challenge was
30 performed with the neat test sample (20.2 % PHMB). Challenge of test and control guinea pigs
31 resulted in signs of mild to moderate erythema in 14 out of 20 test animals and mild erythema
32 in 1 out of 8 controls at 24 hr (net frequency of response of 57.5 %). At 48 hr, mild to
33 moderate erythema was present in 15 out of 20 test animals and mild erythema was still
34 present in 1 control animal (net frequency of response of 62.5 %). Although 1 control showed
35 signs of skin irritation, the test material should be considered as having caused moderate to
36 strong skin sensitisation under the conditions of this study.

37
38 Ref.:58
39
40

41 The possible cross-reactivity of PHMB (Vantocil IB, batch: ADGM 1021, 20 % aqueous PHMB
42 solution) with chlorohexidine gluconate was examined in a study according to the Magnusson
43 and Kligman method using female Dunkin Hartley guinea pigs (20 test and 8 control animals).
44 The design was comparable to OECD 406 with the exception that no SLS was applied during
45 the induction period. Intradermal induction was performed with 0.25 % PHMB in water and
46 0.25 % PHMB in water and FCA. Topical induction (one week later, 48 hr occlusive dressing)
47 was performed with 20 % PHMB. Challenge was performed with 20 % PHMB or 0.05 %, 0.5 %
48 and 4 % of chlorohexidine gluconate. The challenge of test and control guinea pigs with 20 %
49 PHMB resulted in skin findings in 8 out of 20 test animals and in 3 out of 8 control animals
50 yielding a net frequency of response of 2.5 %. No cross reactivity with chlorohexidine
51 gluconate was observed. Rechallenge with 20 % PHMB resulted in positive skin reactions in 3
52 out of 20 test animals. The study authors concluded that PHMB was a mild sensitiser under the
53 conditions of this study.

54
55 Ref.:59
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58

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1 **SCCS comment**

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

5
6
7 Buehler test

8
9
10 Guideline: considered comparable to OECD TG 406
11 Species/strain: Guinea pigs, Alderley Park
12 Group size: 10 test animals, 10 control animals, females
13 Test substance: Vantocil IB (20 % aqueous solution of PHMB)
14 Batch: [BX 791/2 (ADGM 1021)]
15 Purity: no information
16 Vehicle:
17 Concentration: topical induction and challenge: 10 % of material as delivered (2 % a.i.)
18 Rechallenge: 20 %, 10 % and 1 % of material as delivered (4, 2 and 0.2
19 % a.i.)
20 Positive control: /
21 GLP: yes
22 Study period: report dated 1980

23 For induction, animals received topical application of 0.4 ml of test substance (2 % a.i.) or
24 vehicle to the clipped scapular region. The induction site was occlusively covered and the
25 dressing was held in place for 6 hr. Induction with 2 % a.i. was repeated for 10 6-day
26 treatments within 3 weeks. Challenge exposures (2 % a.i.) of 6 hr were performed two weeks
27 after the last induction exposure and reactions were scored 24 and 48 hr after patch removal.
28 Challenge of test and control animals resulted in signs of faint erythema in 6/10 test animals
29 and no erythema in control animals at 48 hr. Rechallenge with 4 % a.i. resulted in faint to
30 moderate erythema in 8/9 test animals and faint erythema in 3/10 controls. Rechallenge with 2
31 % a.i. resulted in faint erythema in 3/10 test animals and no erythema in controls. Rechallenge
32 with 0.2 % a.i. resulted in no response in test and control animals. The study authors
33 considered the test substance as 2 % a.i. as a moderate sensitiser under the conditions of this
34 study. A challenge dose-response was postulated.

Ref.: 58

35
36
37
38 **SCCS comment**

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

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1 Concentration: see Table
 2 Positive control: /
 3
 4 GLP: yes
 5
 6 Study period: report dated 1983
 7
 8

9 The effect of variation of induction and challenge concentrations of PHMB as postulated in the
 10 earlier study was further investigated in a study investigating a variety of induction and
 11 challenge concentration by using the Buehler protocol.
 12
 13 The concentrations used for induction and challenge exposures as well as the results are given
 14 in table 3.
 15
 16
 17

18 Table 3: overview on responses to PHMB under various induction and challenge conditions
 19

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	/ 2.0	1/10 0/10	0/10 0/10	10 0	no

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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 comment

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

General SCCS comment on sensitisation

Based on various guinea pig maximisation- and Buehler tests, PHMB can be considered as a moderate to strong sensitiser in animals. The threshold for eliciting skin reactions OR for elicitation in guinea pigs is approximately 1 %. The SCCS notes that a harmonised classification as Skin Sens 1B H317 has been assigned according to Annex VI of Regulation (EC) No. 1272/2008).

3.3.4 Toxicokinetics

3.3.4.1 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 were excluded (electrical barrier resistance test)
Test substance:	PHMB
Batch	

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1	(labelled test substance):	99BDR99, [¹⁴ C]-PHMB, specific activity: 38.9 mCi/g
2	Purity	
3	(labelled test substance):	9.84 % radiochemical purity (HPLC)
4	Batch	
5	(unlabelled test substance):	09GR24243581, 19.2% aqueous PHMB
6	Purity	
7	(unlabelled test substance):	no information
8	Test item:	Oil/Water emulsion with labelled and non-labelled PHMB
9	Dose level:	2.06 mg/cm ² , Total PHMB applied 18.8 µg
10	Receptor fluid:	phosphate buffered saline (PBS)
11	Method of Analysis:	liquid scintillation counting
12	GLP:	yes (report not signed by the study QA responsible person)
13	Study period:	2014

14
15 Split-thickness skin samples (380 – 400 µm) from 7 donors prepared from frozen, full thickness
16 skin samples were used for the study. Split-thickness skin samples were stored at -20°C in a
17 freezer before use. Samples were mounted onto a static diffusion cell and – after exclusion of
18 skin samples not fulfilling integrity requirements - dosed with [¹⁴C]-PHMB at about 0.3 %
19 [w/w] in a representative cosmetic formulation. A temperature of 32 ± 1°C was maintained in
20 the system: the application rate was 2.0 and 2.11 mg/cm² (target: 2.0 mg/cm²). Receptor
21 fluid was collected prior to dosing and 0.5, 1, 2, 4, 8 and 24 hr after dosing. At the last
22 sampling point, skin samples were washed, dried and removed from the apparatus in order to
23 remove stratum corneum by 20 tape strippings. Epidermis and dermis were then heat
24 separated and all skin samples were analysed by liquid scintillation counting.

25
26 **Results**
27 Only 12 samples from 5 donors (from a total of 19 samples from 7 donors) could be used for
28 evaluation due to processing errors and one sample being considered as an outlier. The mean
29 mass balance was 97.60 % of the applied dose at 24 hr post dose. At the end of the 24 hr
30 exposure period, 49.15 % was washed off. A further 0.54 % of the applied dose was removed
31 from the donor chamber wash leading to a total dislodgeable dose of 49.69 % of the applied
32 dose. The mean total unabsorbed dose was 94.10 % of the applied dose. This consisted of the
33 dislodgeable dose, unexposed skin (0.02 %) and the radioactivity associated with the stratum
34 corneum (44.40 %). The first two tape strips contained 21.84% of the applied dose. There was
35 a steady decrease in the recovery of radioactivity associated with the stratum corneum. Tapes
36 3-5, 6-10, 11-15 and 16-20 contained 11.69 %, 7.02 %, 2.58 % and 1.26 %, respectively.
37 Those amounts retained by the stratum corneum at 24 h were not considered to be dermally
38 absorbed. The absorbed dose (mean: 0.17 %) was the sum of the receptor fluid (0.171 %) and
39 the receptor wash (0.01 %). Dermal delivery (3.49 %) was the sum of the absorbed dose and
40 the portion in the epidermis (3.18 %) and the dermis (0.14 %). The mass balance,
41 dislodgeable dose, unabsorbed dose, absorbed dose and dermal delivery were 5866, 2983,
42 5658, 10.3 and 208 ng equiv./cm², respectively. Due to the relatively low level of absorption,
43 the steady state flux could not be determined. An overview on the results of the dermal
44 absorption study is given in Table 4.

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Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 Table 4: results of the *in vitro* dermal absorption study performed by Toner, 2014
2

Results as percent of applied dose	Mean ± SD	Range
<i>Total dislodgeable dose</i> (skin wash + tissue swab + pipette tip + donor chamber wash)	49.69 ± 15.64	22.74 – 74.33
<i>Unabsorbed dose</i> (total dislodgeable dose + stratum corneum + unexposed skin)	94.10 ± 5.99	79.83 – 102.4
<i>Absorbed dose</i> (receptor fluid + receptor rinse + receptor wash)	0.17 ± 0.05	0.1 – 0.24
<i>Epidermis</i>	3.18 ± 2.05	0.39 – 6.52
<i>Dermis</i>	0.14 ± 0.10	0.03 – 0.31
<i>Dermal Delivery</i> (epidermis+ dermis absorbed dose)	3.49 ± 2.08	0.66 – 6.95
<i>Mass Balance</i>	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
<i>Unabsorbed dose</i> (total dislodgeable dose + stratum corneum + unexposed skin)	5657.61 ± 202.79	5254.77 – 6001.88
<i>Absorbed dose</i> (receptor fluid + receptor rinse + receptor wash)	10.33 ± 3.06	6.05 – 14.56
<i>Epidermis</i>	189.40 ± 120.93	23.84 – 402.51
<i>Dermis</i>	8.21 ± 6.08	1.61 – 20.34
<i>Dermal Delivery</i>	207.94 ± 123.77	40.93 – 428.89

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

(epidermis+ dermis+absorbed dose)		
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 $207.94 + 247.54 \text{ ng equiv./cm}^2 = 455.48 \text{ ng equiv./cm}^2$.

Ref.: 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): [¹⁴C] PHMB
 Radiochemical reference no.: 4602
 Specific activity: 1.85 GBq/732 mg
 Dose volume: 10 and 200 µl/cm²
 Receptor fluid: distilled water
 Method of Analysis: liquid scintillation counting
 GLP: yes
 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

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 glass diffusion cells (exposure area: 2.54 cm²) and placed in a water bath maintained at 30 ±
2 1 °C. Only membranes with a calculated resistance > 10 kΩ were used.

3 Admixture of labelled compound to unlabelled compound was performed to achieve activities
4 between 3 x 10⁶ and 4 x 10⁹ dpm/ml. Nominal concentrations of 200.0, 20.0 and 2.0 g/l were
5 applied at 10 µl/cm² and left unoccluded whereas the concentration of 200 g/l was also applied
6 at 200 µl/cm² and kept occluded. At recorded time intervals, 250 µl samples of receptor fluid
7 were taken for analysis; the volume removed from the receptor chamber was replaced by
8 addition of the same volume of receptor fluid. The total observation period was 96 hrs.

9
10
11 **Results:**

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

13

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

14
15
16 **SCCS comment**

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

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

Ref.: 19

1
2
3
4 Guideline: /
5 Species/strain: Human skin
6 Membrane
7 integrity: membranes with a calculated resistance < 10 kΩ were
8 excluded
9 Number of donors: not stated
10 Method: analysis of receptor fluid in glass diffusion cells
11 Test substance
12 (unlabelled): PHMB from Zeneca Inc. Dighton, Mass., USA; 20.2 % aqueous solution
13 Batch: no data
14 Purity: no information
15 Test substance
16 (labelled): [14C] PHMB
17 Radiochemical
18 reference no.: 1581
19 Specific activity: 1.4 MBq/mg
20 Dose volume: 200 µl/cm²
21 Receptor fluid: distilled water
22 Method of
23 Analysis: liquid scintillation counting
24 GLP: yes
25 Study period: 1998

26
27 Epidermal membranes from human skins stored frozen (no further information on donors, skin
28 location, number of individual donors) were used for the study intended to investigate skin
29 penetration at elevated temperature (spa conditions). Skin samples were mounted on glass
30 diffusion cells (exposure area: 2.54 cm²) and placed in a water bath maintained at 40 ± 1 °C.
31 Only membranes with a calculated resistance > 10 kΩ were used. Admixture of labelled
32 compound to unlabelled compound was performed to achieve activities between 3 x 10⁸
33 dpm/ml. A 20 % dosing solution was warmed to 40°C and applied at 200 µl/cm² to two groups
34 of skin membranes. After application, one group was occluded for 0.5 hrs and then washed
35 with 3 % Teepol® and distilled water and the kept unoccluded until 24 hr after application. The
36 other group of membranes was kept occluded for 24 hr after substance application. At recorded
37 time intervals, 250 µl samples of receptor fluid were taken for analysis; the volume removed
38 from the receptor chamber was replaced by the addition of the same volume of receptor fluid.
39 The total observation period was 96 hrs.

40
41 Results

42
43 Table 5: *in vitro* dermal absorption of [14C] PHMB in human skin

44

Test condition [concentration, applied rate, occlusion]	Time period [hr]	Absorption rate [µg/cm ² /hr]	Absorption Percentage [%]
200 g a.i./l	0 - 24	< 0.002 ± < 0.001	< LOQ

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

200 µl/cm ² occluded for 0.5 hr, then unoccluded for 23.5. hrs [n=6]			
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
Alderley-Park rats (male and female)
Membrane integrity: permeability constants (tritiated water) > 1.3 x 10⁻³ cm/hr
(human skin) and > 1.5 x 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): ¹⁴C- PHMB, slightly higher than 20% aqueous solution
Batch: CTL Radiochemical Card 878
Specific activity: 0.88 mCi/ml
Dose volume: 1 ml
Receptor fluid: sterile physiological saline
Method of Analysis: LSC
GLP: yes
Study period: 1982

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

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 Human and rat skin samples were mounted on glass diffusion cells. After checking integrity, 1
2 ml of PHBM solution (17.6 μCi) was added to the donor chamber in the wet experiments. At
3 least 2 x 25 μl receptor fluid samples were taken daily for up to 15 days. In dried-on
4 experiments, skin was exposed to atmosphere overnight before the addition of 36 μl 20 % ^{14}C
5 PHMB (31.7 μCi). 25 μl samples were taken daily until slow absorption rate became apparent.
6 Furthermore, an uptake experiment was performed in which 2 cm^2 skin disks were taken into
7 "bathing" solutions of different PHMB concentrations. After an equilibration phase of 5 days,
8 skin samples were weighed and dissolved and partition coefficients were determined.
9 Under dried-on conditions, absorption of PHBM through human skin was not measurable,
10 which might in part be due to the limits of detection (absorption from aqueous solutions could
11 not be measured below 0.4 % ^{14}C PHMB). Steady state of absorption rates achieved under
12 wet conditions are presented in the Table 6. The LOQ and the low absorption rate complicated
13 the determination of a permeability constant; however, a value of $5 \times 10^{-6} \text{ cm hr}^{-1}$ was
14 suggested by the study authors.
15
16
17

18 Table 6: *in vitro* dermal absorption of [^{14}C] PHMB in rat and human skin.
19

20 Skin sample	21 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

23
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25
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27 **SCCS comment**

28 Skin samples are poorly described; there is no information on the number of donors. The
29 number of skin samples analysed per donor is also not stated. The surface area exposed to the
30 test item is not known. Recovery was not reported. The study was not performed according to
31 current guidelines. Results of this study cannot be considered to derive a realistic dermal
32 absorption rate of PHMB.
33

34 Ref.:28
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Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 There also exists a previously performed comparative penetration study of lower reliability.
2 Radiolabelled PHMB ($[^{14}\text{C}]$ -Vantocil, no further information) was applied to skin biopsies of
3 newborn, hairless rats. The skin biopsy samples were punched and placed in a chamber filled
4 with the medium on a perforated plate of stainless steel placed on filter paper. PHMB was
5 applied as 5 % solution and samples were taken during the first 12 hr at one-hr intervals and
6 thereafter 3 samples were taken daily during an 8-hr period for the following 4 consecutive
7 days. In comparison, four biopsies of human skin were punched, the epidermis was separated
8 and treated as in the part with rat skin biopsies with the exception that after 48 hr, 1 ml DMSO
9 was added. The radioactivity was measured by means of a liquid scintillation counter. In the
10 study part using rat skin biopsies, no skin absorption was detected up to day 5 of exposure. In
11 contrast, in human epidermal skin biopsies a low rate of penetration of about 0.09 % was
12 noted after 24 hr and this penetration rate was from 0.11 % up to 0.81 % after adding DMSO.
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14 Ref.: 61

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18 **SCCS comments**

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

28 24- and 72-hours *in vitro* percutaneous absorption

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30	Guideline:	OECD 428 (2004); SCCS/1358/10 adopted 22 June 2010; COLIPA
31		Guidelines for Percutaneous Absorption/Penetration (1997)
32	Test system:	frozen dermatomed human skin
33	Membrane	
34	integrity:	transcutaneous electrical resistance > 4 kΩ
35	Replicates:	12 for each study (4 donors)
36	Method of analysis:	liquid scintillation counting
37	Test substance	
38	(unlabelled):	PHMB (Polyhexamethylene Biguanide)
39	Batch:	14GR177464 (non-radiolabelled), CFQ42591 (radiolabelled)
40	Purity:	> 94.2 % (w/w), non-radiolabelled
41	Test item:	0.1% (w/w) $[^{14}\text{C}]$ -PHMB in Test preparation I (aqueous micellar solution)
42		and in Test Preparation 2 (oil in water emulsion) for 24-h study;
43		0.3% (w/w) $[^{14}\text{C}]$ -PHMB in Test preparation I (aqueous micellar solution)
44		and in Test Preparation 2 (oil in water emulsion) for 72-h study;
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46	Dose volume:	200 µl/cm ²
47		
48	Exposed area:	3.14 cm ²
49	Receptor fluid:	phosphate buffered saline (PBS) with sodium azide (0.01%, w/v)
50	Stability in	
51	Receptor fluid:	/
52	Tape stripping:	5 tape strips

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1 GLP: in compliance
2 Study period: December 2015- March 2016
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6 Human abdominal skin samples were obtained from four different donors. The skin was
7 dermatomed (370 - 400 μm) and then the split-thickness membranes stored frozen, at
8 approximately -20°C , wrapped in aluminium foil until use. Dermatomed skin membranes (12
9 skin membranes from 4 donors) were thawed and checked for integrity by the transcutaneous
10 electrical resistance method prior to use. The skin samples exhibiting a resistance less than 4
11 $\text{k}\Omega$ were excluded.

12 [^{14}C]-PHMB was applied in two test preparations in Test preparation I (aqueous micellar
13 solution) and in Test Preparation 2 (oil in water emulsion) to human split-thickness skin
14 membranes (3.14 cm^2) mounted in static diffusion cells *in vitro*. The test preparation was
15 applied at a target application rate of *ca* 2 mg/cm^2 for 24 h and in the second study for 24 h
16 with an extended post exposure monitoring period of 72 h. Absorption of PHMB was evaluated
17 by collecting receptor fluid aliquots (300 μL) at predose, 1, 2, 4, 6, 8 and 12 h post dose. An
18 additional sample at 24 and 48 h post dose was taken for an extended observation study. At 24
19 h post dose, the skin was washed with an aqueous solution of polysorbate 20 (2%, w/v) and
20 water. The skin was then dried with tissue paper swabs. The skin was then removed from the
21 cells, dried and the upper stratum corneum removed by tape stripping (5 tape strips). The
22 remaining skin was divided into exposed and unexposed skin. Then the exposed epidermis was
23 separated from the dermis by heat separation. All samples were analysed by liquid scintillation
24 counting.
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26 Results

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28 A summary of the mean results obtained in the 24-h study is provided in the Table below.
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Test Preparation	Test Preparation 1 Aqueous micellar solution	Test Preparation 2 Oil/water emulsion
Target [^{14}C]-PHMB Concentration in Test Preparation (% w/w)	0.1	0.1
Actual [^{14}C]-PHMB Concentration in Test Preparation (% w/w)	0.11	0.10
Target Application Rate of Test Preparation (mg/cm^2)	2	2
Actual Application Rate of Test Preparation (mg/cm^2)	1.97	1.97
Total Number of Donors	4	4
Total Number of Replicates Dosed	12	12
Total Number of Replicates Contributing to Mean \pm SD Data	12	11
(% Applied Dose)	(Mean \pm SD)	(Mean \pm SD)
Total Dislodgeable Dose*	48.43 \pm 8.32	52.35 \pm 24.18
Upper Stratum Corneum (Tapes 1-5)	29.33 \pm 5.46	31.27 \pm 16.19
Unabsorbed Dose **	77.88 \pm 6.58	83.70 \pm 11.11
Epidermis + Lower Layers of Stratum Corneum	11.47 \pm 5.69	14.20 \pm 8.07
Dermis	1.56 \pm 2.49	1.02 \pm 0.84
Receptor Fluid	0.01 \pm 0.01	0.02 \pm 0.03
Receptor Chamber Wash	0.02 \pm 0.01	0.02 \pm 0.01
Absorbed Dose ***	0.03 \pm 0.01	0.04 \pm 0.04
Mass Balance	90.93 \pm 2.11	98.96 \pm 5.44
(ng equiv./cm^2)	(Mean \pm SD)	(Mean \pm SD)
Total Dislodgeable Dose*	1007 \pm 173	1073 \pm 496

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Upper Stratum Corneum (Tapes 1-5)	610 ± 113	641 ± 332
Unabsorbed Dose **	1620 ± 137	1716 ± 228
Epidermis + Lower Layers of Stratum Corneum****	238 ± 118	291 ± 165
Dermis	32.3 ± 51.9	20.9 ± 17.2
Receptor Fluid	0.25 ± 0.12	0.37 ± 0.54
Receptor Chamber Wash	0.33 ± 0.20	0.35 ± 0.30
Absorbed Dose ***	0.58 ± 0.25	0.72 ± 0.81
Mass Balance	1891 ± 43.9	2028 ± 111

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

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

*** Absorbed dose = receptor fluid + receptor wash

**** Epidermis + Lower Layers of Stratum Corneum = Epidermis + Lower Layers of Stratum Corneum + cling film

Notes

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

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

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

A summary of the mean results obtained in the 72h (extended monitoring time) study is provided in the Table below.

Test Preparation	Test Preparation 1 Aqueous micellar solution	Test Preparation 2 Oil/water emulsion
Target [¹⁴ C]-PHMB Concentration in Test Preparation (% w/w)	0.3	0.3
Actual [¹⁴ C]-PHMB Concentration in Test Preparation (% w/w)	0.34	0.32
Target Application Rate of Test Preparation (mg/cm ²)	2	2
Actual Application Rate of Test Preparation (mg/cm ²)	1.96	2.00
Total Number of Donors	4	4
Total Number of Replicates Dosed	12	12
Total Number of Replicates Contributing to Mean ± SD Data	12	12
(% Applied Dose)	(Mean ± SD)	(Mean ± SD)
Dislodgeable Dose 24 h	53.33 ± 7.70	58.10 ± 18.33
Total Dislodgeable Dose*	54.23 ± 7.85	59.90 ± 17.47
Upper Stratum Corneum (Tapes 1-5)	22.58 ± 3.98	23.24 ± 11.84
Unabsorbed Dose **	76.92 ± 7.44	83.31 ± 7.70
Epidermis + Lower Layers of Stratum Corneum	14.54 ± 8.18	14.45 ± 6.63
Dermis	1.23 ± 0.85	1.46 ± 1.67
Receptor Fluid	0.01 ± 0.01	0.02 ± 0.01
Receptor Chamber Wash	<0.01 ± <0.01	0.01 ± 0.01
Absorbed Dose ***	0.02 ± 0.01	0.03 ± 0.02
Mass Balance	92.71 ± 1.64	99.25 ± 5.89
(ng equiv./cm ²)	(Mean ± SD)	(Mean ± SD)
Dislodgeable Dose 24 h	3566 ± 515	3706 ± 1169
Total Dislodgeable Dose*	3626 ± 525	3821 ± 1114
Upper Stratum Corneum (Tapes 1-5)	1510 ± 266	1482 ± 755
Unabsorbed Dose **	5143 ± 497	5314 ± 491
Epidermis + Lower Layers of Stratum Corneum****	972 ± 547	921 ± 423
Dermis	82.0 ± 57.0	93.4 ± 107
Receptor Fluid	0.97 ± 0.50	1.13 ± 0.90
Receptor Chamber Wash	0.31 ± 0.22	0.81 ± 0.95
Absorbed Dose ***	1.29 ± 0.60	1.94 ± 1.52
Mass Balance	6199 ± 110	6331 ± 375

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

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1 As no movement of PHMB from the skin reservoir to the receptor fluid occurred between
2 24 and 72 hours, SCCS is willing to accept that the amount in the epidermis may be
3 excluded as dermally absorbed. The amount present in the living epidermis after 5
4 strippings still represents 11-15%.

5
6 **General SCCS comment on percutaneous penetration**

7 From previous submissions

8
9 Four studies on percutaneous penetration of PHMB were not in compliance with SCCS
10 requirements, qualities of the studies were compromised by poor sample description and the
11 studies have not been performed with representative cosmetic formulations containing 0.3 %
12 PHMB. After the commenting period which followed the publication of SCCS/1535/14 on 18
13 June 2014, a draft of a new *in vitro* percutaneous absorption study was provided by the
14 applicant.

15
16 The latter dermal penetration study was performed according to OECD TG 428 and SCCS basic
17 requirements for dermal penetration studies and was in line with GLP principles. Despite some
18 processing errors and an outlier and despite the fact the study was still in the state of a draft
19 report it was considered acceptable for determination of dermal absorption of PHMB.

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

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

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33 Submission III

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35 In the new submission, dermal absorption studies for 0.1% and 0.3% PHMB in two different
36 representative cosmetic formulations (aqueous micellar solution, o/w emulsion) were
37 submitted. Measurements of penetration into receptor fluid were done at 24 h (0.1%) up to 72
38 h (0.3%). Additionally, the number of tape strips was diminished from 20 to 5 in response to
39 previous comments by the SCCS on submission II.

40 The quality of the barrier function of the skin samples was assessed by TER measurement. The
41 chosen cut-off value of 4 k Ω is not common practice (threshold of 10 k Ω is normally used) and
42 may lead to an overestimation of the results.

43
44 After 72h, 0.01% of PHMB-derived radioactivity migrated to the receptor fluid. Therefore the
45 SCCS agrees that the amount found in the epidermis may be excluded from the total absorbed
46 dose, according to SCCS/1564/15 (*In the case of substances with very low dermal absorption
47 and limited permeation (e.g. colourants or UV-filters with high molecular weight and low
48 solubility), the epidermis may be excluded when it is demonstrated that no movement of the*

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 *chemicals from the skin reservoir to the receptor fluid occurs (Yourick et al., 2004; WHO,*
2 *2006). Adequate detection of substances poorly soluble in water is important in the receptor*
3 *fluid of in vitro dermal absorption study to ascertain that the dermal absorption concerns the*
4 *active substance and not the impurities').*

5
6 Since the new absorption studies were performed according to the SCCS guidelines, one SD
7 was added for the final calculation of the dermal absorption value. Consequently, the value of
8 4.09 % will be used for the MoS calculation (1.56% dermis + 0.03% total absorbed + 2.5% 1
9 SD).

10
11 The value found using the new dermal absorption study, being 4.09%, is in line with
12 information received via ECHA. According to the cited guidance (Guidance on Dermal
13 Absorption, EFSA Panel on Plant Protection Products and their Residues (PPR), EFSA 2012), a
14 rough estimation of dermal absorption of around 8.5% could be proposed.
15 From RAC information (Committee on Risk Assessment RAC, background Document to the
16 Opinion proposing harmonised classification and labelling at Community level of
17 Polyhexamethylene biguanide or Poly(hexamethylene) biguanide hydrochloride or PHMB,
18 2011), the dermal absorption was estimated to be 4 %.

21 3.3.4.2 Other studies on toxicokinetics

22 /

27 **3.3.5 Repeated dose toxicity**

30 3.3.5.1 Repeated Dose (28 days) oral / dermal / inhalation toxicity

33 ORAL

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

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

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10 Results

11 One male and one female animal receiving the highest dose were killed on day 4 due to
12 bodyweight loss and deterioration of clinical condition. Dose-related loss in bodyweight/body
13 weight gain and reduced water and/or food consumption predominantly occurring during the
14 first days of treatment (and recovery thereafter) were considered as a palatability effect.
15 Further treatment-related findings consisted of increases in plasma cholesterol levels and ALP
16 and AST activities at the highest dose tested, increased liver weight at 1 mg/ml and decreased
17 liver weight at 2 mg/ml and a dose-related increase in kidney weight at all dose levels. No
18 NOAEL could be derived from the study. A highest dose level of 1.0 mg/ml was recommended
19 for the 2-year drinking water study. 0.1 mg/ml should be considered as the LOAEL based on
20 the results from this study.
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23 Ref.: 47
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26 Guideline: / (range-finding study for 2-year drinking water study)
27 Species/strain: Mouse, C57Bl/10JfAP/Alpk
28 Group size: 10/sex/dose
29 Test substance: PHMB, 20 % aqueous solution
30 Batch: D4097
31 Purity: no details given
32 Vehicle: sterilised water
33 Dose levels: 0, 0.1, 0.3, 0.6, 1.2 mg/ml
34 Route: oral
35 Administration: drinking water
36 Duration: 28 days
37 GLP: no statement
38 Study period: 1991
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47 The stability of the test sample in drinking water was determined prior to start. Individual
48 clinical observations were recorded daily. Individual bodyweights and food consumption per
49 cage (5 animals/ cage) were recorded daily for the first week and weekly thereafter. After 28
50 days of treatment, all surviving animals were killed and subjected to gross post mortem
51 examination. Liver and kidney weights were recorded and blood samples were obtained from
52 each animal.
53

54 Results

55 One male animal of the 0.3 mg/ml group was found dead on day 13. Dose-related initial loss of
56 body weight, reduction in food and water consumption and continued reduction in body weight
57 and water consumption were considered to be due to palatability of the substance. Changes
58 considered to be treatment-related included a decrease in plasma ALT activity and a decrease

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1 in liver weight for males given 0.6 and 1.2 mg/ml and were most probably associated with the
2 poor nutritional status. As effects on bodyweights and water consumption were present at all
3 dose levels, a NOAEL could not be derived. A highest dose level of 0.3 mg/ml was
4 recommended for the 2-year drinking water study. 0.1 mg/ml should be considered as the
5 LOAEL based on the results from this study.

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Ref.: 48

Dermal

Guideline: / (similar to OECD TG 410)
Species/strain: Rat, Alp: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 were 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

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1 rabbits were killed. Blood was taken for haematological examination and livers, adrenals,
2 kidneys, spleens, ovaries, uteri, hearts, thymuses and lungs were examined
3 histopathologically.
4 No signs of systemic toxicity or skin irritation were noted during the experimental period and
5 the animals gained body weight at the same rate as control animals. There were no signs of
6 organ damage at autopsy and histopathological examination of the tissues did not reveal any
7 changes attributable to treatment. Haematological findings indicated no significant differences
8 between test and control animals (reference: Elks and Hope, 1972).
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10 Ref.: 32
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14 Inhalation

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16 Guideline: OECD TG 412
17 Species/strain: Rat, Alpk:APfSD (Wistar-derived)
18 Group size: 5/sex/dose (group)
19 Test substance: PHMB, 19.2 % [w/w] aqueous solution
20 Batch: BX 6142
21 Purity: HMD < 0.1 %; HMBDA 0.1 %
22 Dose levels: 0; 0.025; 0.25; 2.5 µg/l [mg/m³]
23 Vehicle: deionised water
24 Route: inhalation
25 Administration: nose-only: 6 hr/day; 5 days/week
26 Duration: 28 days (13 week recovery for satellite groups at 0; 0.025; 2.5 µg/l)
27 GLP: yes
28 Study period: 2003 - 2004, report dated 2006
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36 The study was preceded by two 5-day range-finding studies in rats. In these studies, dose-
37 dependent histopathological findings in the lung and larynx were observed, indicative of
38 respiratory irritation.

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40 Animals were exposed as indicated. Target concentrations of aqueous solutions of 0 (control),
41 0.025, 0.25 or 2.5 mg/m³ corresponded to PHMB concentrations of 0.0239 mg/m³ (MMAD
42 range – 0.32-1.30 µm), 0.257 mg/m³ (MMAD range – 0.48-5.06 µm) and 2.47 mg/m³ (MMAD
43 range – 0.67-1.67 µm). Bodyweights were measured weekly and food consumption was
44 measured continuously throughout the study. At the end of the scheduled period, the animals
45 were killed and examined by gross pathology. Cardiac blood samples were taken for
46 haematology and clinical pathology, selected tissues were weighed (adrenal glands, ovaries,
47 epididymis, spleen, kidneys, testes, liver, thymus, lungs (with trachea attached but larynx
48 removed), brain, heart, uterus and cervix). Tissues were almost completely taken and
49 processed for subsequent histopathology.
50

51 Results

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54 There were no treatment-attributable deaths and clinical signs up to 2.5 mg/m³ (clinical
55 observations during exposure and post-exposure were attributed to the restraint nose-only
56 conditions). Body weights were lower than for controls in males exposed to 0.25 mg/m³ or 2.5
57 mg/m³. Following cessation of exposure there was some recovery in body weight for males at
58 2.5 mg/m³. Food consumption was slightly low in weeks 2 and 4 for males exposed to 0.25 and

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1 2.5 mg/m³. There were no changes in haematology or blood clinical chemistry parameters that
2 were of toxicological significance.
3
4 Lung weights were slightly elevated for males and females exposed to 2.5 mg/m³ and thymus
5 weights were slightly elevated in males only at 2.5 mg/m³. No macroscopic treatment-related
6 findings were observed at post-mortem examination.
7 Treatment-related microscopic findings were recorded in the larynx, trachea and lungs. On
8 completion of the 28-day exposure period, squamous metaplasia was seen in the larynx of
9 males and females at 0.25 and 2.5 mg/m³, and tracheal inflammation for males and females at
10 2.5 mg/m³. Comparable findings were absent 13 weeks following cessation of treatment for
11 animals previously exposed to 2.5 mg/m³. Pneumonitis and bronchitis in the lung were seen for
12 males and females exposed to 2.5 mg/m³, both at end of the exposure period and at the end
13 of the recovery period. The severity of pneumonitis was slightly reduced at the end of the
14 recovery period. The higher thymus weight for males only exposed to 2.5 mg/m³, in the
15 absence of any histopathological changes, was considered to be of unknown toxicological
16 significance.
17
18 PHMB exposure to concentrations of 0.257 or 2.47 mg/m³ resulted in some transient
19 histopathological changes in the larynx and trachea that were characteristic of exposure to a
20 respiratory tract irritant. These changes were clearly reversible following cessation of exposure.
21 Some bodyweight changes were also present at these exposure concentrations. Some non-
22 resolving histopathology changes in the lungs (pneumonitis and bronchitis) were limited to the
23 highest exposure concentration of 2.47 mg/m³. A NOAEL of 0.0239 mg/m³ was derived from
24 the study.

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26 Ref.: 76, 77, 78
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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/m³
Route: inhalation
Administration: snout-only: 6 hr/day; 5 days/week
Duration: 3 weeks
GLP: /
Study period: 1976

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31 Animals were exposed as indicated to atmospheres of respirable particles (MMAD < 7 µm).
32 Body weight, food and water intake and signs of gross toxicity were checked daily. After the
33 last exposure, blood and urine samples were obtained, animals were killed and selected tissues
34 (lung, trachea, thymus, liver, kidneys, adrenals, spleen, heart, gonads, epididymes, uteri) were
35 taken for histopathological examination.
36

37 Results:

38 There were no signs of toxicity at the lowest dose tested. At 0.25 mg/m³ one animal died after
39 the 13th exposure and animals did not gain weight or even lost weight. The experiment was

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1 terminated after 13 exposures. Animals showed signs of moderate nasal irritation and
2 tachypnoea. There were significant amounts of methaemoglobin in all animals (5, 4 and 4% in
3 males and 3, 7, 5 and 3% in females). Histopathological examination of stained sections
4 revealed slight to moderately severe pneumonitis. There was also evidence of accompanying
5 resolution of the lung lesions in all the affected animals. The thymuses of 3 male and 3 female
6 rats from the test group showed reduction in the cortical thickness and depletion of
7 lymphocytes. Patchy loss of cilia in the tracheal epithelium was observed in three animals. The
8 testis of one male showed degeneration of a few seminiferous tubules. At 2.75 mg/m³ animals
9 showed signs of nasal irritation and dyspnoea and dramatic body weight loss after the 4th
10 exposure. The experiment was terminated after the 6th exposure. There were significant
11 increases in methaemoglobin in all animals. Histopathological examination of tissues revealed a
12 moderate to severe pneumonitis. The thymus glands showed severe depletion of lymphocytes
13 and loss of normal architecture. Exposure to 12.5 mg/m³ resulted in severe nasal irritation and
14 dyspnoea. During the first three days of exposure, all animals lost weight and their intake of
15 food and water was very low. One female rat died towards the end of the fourth exposure and
16 the remainder died overnight. Exposure of rats to 26.0 mg/m³ resulted in severe nasal
17 irritation and dyspnoea. During the first three days of exposure all animals lost weight and their
18 intake of food and water was very low. As in the test with 12.5 mg/m³ exposure, one female
19 rat died towards the end of the fourth exposure and the remainder died overnight. A NOAEL of
20 0.025 mg/m³ was derived from this study.

Ref.: 15

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26 **SCCS Comments on repeat-dose inhalation studies**

28 Based on the severity of the effects caused by inhalation of PHMB, the absence of reversibility
29 of inflammation in the respiratory tract and the very low doses causing these effects, a
30 harmonised classification as STOT RE 1 H372 (causes damage to the respiratory tract through
31 prolonged or repeated exposure by inhalation) has been assigned according to Annex VI of
32 Regulation (EC) No. 1272/2008

33
34 According to the procedures of classification of mixtures, classification with respect to STOT-RE
35 is not warranted if the substance classified as STOT-RE 1 is present at concentrations below
36 1% in the mixture. See ECHA Guidance on the application of CLP criteria (SCCS Ref. III).

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38
39

40 3.3.5.2 Sub-chronic (90 days) oral / dermal / inhalation toxicity

41
42
43
44

ORAL

45 The subchronic toxicity of Vantocil P (20.2 % aqueous PHMB solution, Batch D4097) was
46 investigated in 90 d dietary studies in mice (C57Bl/10JfCD- 1) and rats (Alpk:APfSD, Wistar
47 derived; 12/sex/dose with 4 animals/sex/group used for interim sacrifices at 29 days) intended
48 as range-finders for chronic toxicity studies. The studies were in accordance with GLP but not
49 strictly in accordance with OECD TG 408. Dose levels in rats were 0, 1000, 2000, 4000, 6000
50 ppm a.i. (corresponding to about 0, 83.9, 171.5, 373.0, 556.1 mg/kg bw/day a.i. in males and
51 92.3, 192.9, 409.8, 617.4 mg/kg bw/day a.i. in females). In mice, dose levels were 0, 1000,

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1 2000, 4000 ppm a.i. (corresponding to about 0, 162, 328, 736 nmg/kg bw/day a.i. in males
2 and 0, 224, 445, 963 mg/kg bw/day a.i. in females) and 6000 ppm a.i. (terminated due to high
3 mortality).
4

5 Results in rats:

6 Dietary administration of PHMB led to a treatment-related reduction in body weight at \geq 2000
7 ppm accompanied by clinical-chemical indication of poor nutritional state. From 2000 ppm
8 there were findings in some haematological parameters, notably increased haemoglobin and
9 haematocrit in males. The kidney was identified as a target organ. Renal functional change in
10 the form of decreased urine volume and increased specific gravity was noted at doses of 2000,
11 4000 or 6000 ppm for interim and terminal kill animals, being more marked in males.
12 Histopathological changes were recorded at day 29 at the highest dose. At terminal kill, a clear
13 treatment-related increase in kidney weight was apparent for males at 4000 or 6000 ppm, but
14 histopathology of kidneys to assess the toxicological significance of these weight changes was
15 not performed at terminal kill. Treatment-related increases in plasma alkaline phosphatase,
16 alanine transaminase and/or aspartate transaminase activities were seen at all dose levels for
17 males and for females which received 1000, 2000, 4000 or 6000 ppm at interim and/or
18 terminal kill. As only limited histopathological examination was performed, the toxicological
19 significance of macroscopic changes in the gastrointestinal tract, liver and hepatic lymph node
20 in some terminal kill animals at 6000 ppm could not be assessed. It was concluded that a dose
21 level of 2000 ppm would be suitable for use as the high-dose level in a long-term dietary study.
22 The authors considered 1000 ppm (corresponding to 83.9 mg/kg bw/d in males and 92.3
23 mg/kg bw/d in females) as NOAEL.
24
25
26

27 Results in mice:

28 There were treatment-related effects on initial food consumption in males. Reduced body
29 weights were noted in males at 2000 ppm and marked effects on body weight gain were
30 observed in males and females at 4000 ppm. The highest dose caused marked toxicity leading
31 to termination of this group by day 11. There were no treatment-related effects on liver and
32 kidney weights and no gross or histopathological findings. 4000 ppm was considered as the
33 highest dose to be applied in a long-term feeding study. The authors considered 1000 ppm
34 (corresponding to 162 mg/kg bw/d in males and 224 mg/kg bw/d in females) as the NOAEL of
35 this study.
36
37

38 Ref.: 50, 51
39
40

41 An older, non-guideline compliant 90-day dietary study was performed with antibacterial 9073
42 (25 % aqueous solution of PHMB; Batch WEM/G/680) in Alderley Park Wistar Rats at dose
43 levels of 0, 625 and 1250 ppm a.i. There was no mortality at any dose level. The dietary
44 administration of 1250 ppm led to a retardation of body weight gain most likely due to reduced
45 food consumption due to decreased palatability. At this dose level, deposits of an iron-pigment
46 in the liver (in liver cells and in Kupffer cells) was noted in individual female rats. The dose
47 level of 625 ppm was tolerated without any finding.
48

49 Ref.: 41
50
51

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

10 Ref.: 42

11
12
13 In a poorly reported 90-d drinking water study performed with a 20 % aqueous solution of
14 PHMB (batch D4097) in mice (C57BL/10JfAP/Alpk), only the effect on body weight and
15 macroscopy was investigated. A control and a dose group were used consisting of 10 males and
16 females, respectively. The treatment group received 0.1 mg/ml test substance in the first
17 week, 0.3 mg/ml in the second week and 0.3 mg/ml from the third week until study
18 termination. Administration of PHMB caused a reduction in bodyweight gain of treated animals
19 and a dose-related reduction in water consumption. There were no treatment-related
20 macroscopic post-mortem findings.

22 Ref.: 49

25 3.3.5.3 Chronic toxicity

26
27
28 Guideline: considered mainly in accordance with OECD TG 452

29 Species/strain: Dog/Beagle

30 Group size: 4/sex/dose

31 Test substance: PHMB ASG9112994, 20.2 % aqueous solution

32 Batch: D40987

33 Purity: impurities < 0.76 %

34 Dose levels: 0, 300, 1500, 4500 ppm up to weeks 11/12 and reduced to 3000 ppm
35 thereafter (corresponding to about 0, 11, 54, 169/108 mg/kg bw/day)

36 Route: oral

37 Administration: diet

38 Exposure: daily for 1 year

39 GLP: no statement

40 Study period: 1993 – 1994, report dated 1995

41
42 Dose levels were selected based on the findings of two short-term range-finding studies.

43 Animals received PHMB as dietary admixture. The high dose of 4500 ppm was reduced to 3000
44 ppm from weeks 11/12 onward due to unexpectedly severe reactions (see results) leading to
45 the intercurrent killing of 3 high dose males. The animals were examined for mortality and
46 clinical signs including unusual behaviour. Body weight and food consumption were determined
47 at regular intervals. Blood samples for haematology and clinical pathology were taken in weeks
48 -1, 4, 13, 26 and 52 and from intercurrent high dose males. Urine samples were taken prior to
49 the start of the study and in weeks 26 and 52. All dogs surviving to termination and those
50 killed intercurrently were subjected to a complete necropsy. Adrenal glands, brain, epididymis,

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1 kidneys, liver, testes and thyroid glands were weighed. A comprehensive number of organs and
2 tissues were processed and examined by histopathology.

3
4 **Results**

5 High-dose males exhibited unexpected signs of toxicity including marked reddening/peeling of
6 scrotal skin, loss of appetite, body weight loss and/or indications of liver impairment in the
7 form of elevated plasma alanine transaminase and/or aspartate transaminase activities.

8 Following reduction of the high-dose level to 3000 ppm, one female was killed after showing
9 clinical signs of toxicity. For animals surviving to termination, treatment related red/brown
10 staining of coat, predominantly paws and hocks, present in one female receiving 300 ppm and
11 in all animals at 1500 or 3000 ppm were considered of no toxicological relevance as there were
12 no associated histopathological changes. Plasma cholesterol levels were reduced for the
13 surviving animals receiving 3000 ppm whereas plasma alanine transaminase and/or aspartate
14 transaminase activities were elevated for the surviving high dose male, for 2/3 females
15 receiving 3000 ppm and for one female each at 300 or 1500 ppm. Low testes weight was also
16 apparent for the surviving male at 3000 ppm.

17 Treatment-related histopathological findings were present in the skin (dermatitis of the
18 scrotum, chin and limbs) as well as in the liver, kidney (males only) and testes of animals that
19 received 4500/3000 ppm.

20 There were no histopathological changes in animals receiving 300 or 1500 ppm that were
21 considered attributable to treatment. The elevated plasma alanine transaminase and aspartate
22 transaminase activities in isolated animals receiving 300 or 1500 ppm, in the absence of any
23 associated histopathological changes, were considered to be of no toxicological importance. The
24 study authors considered 1500 ppm (54 mg/kg bw/d) as the NOAEL of the study.

25 Ref.: 52, 81, 82

26
27 **SCCS comment**

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

29
30
31
32 Guideline: Considered comparable to OECD TG 453
33 Species/strain: Rat/Alpk:APfSD (Wistar derived)
34 Group size: 64/sex/dose (12 animals/sex/dose used for interim kill)
35 Test substance: Vantocil P ASG9112994, 20.2 % aqueous solution
36 Batch: D4097
37 Purity: impurities < 0.76 %
38 Dose levels: 0, 200, 600, 2000 ppm (corresponding to about 0, 12.1, 36.3, 126.1
39 mg/kg bw/day in males, 0, 14.9, 45.3, 162.3 mg/kg bw/day in females)
40 Route: oral
41 Administration: diet
42 Exposure period: 104 weeks
43 GLP: yes
44 Study period: 1992 – 1994, report dated 1996
45

46 Animals received diet containing PHMB as indicated and were examined daily for mortality,
47 clinical signs and unusual behaviour. Body weight and food consumption were determined at
48 regular intervals, ophthalmology was performed. Blood samples for haematology and clinical
49 pathology were collected in weeks 14, 27, 53, 79 and 105. Urine samples were collected in

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1 weeks 13, 26, 52, 78 and 104. Animals were subjected to complete necropsy when killed, a
2 comprehensive number of organs and tissues were processed and examined by histopathology.
3

4 Results

5 At 2000 ppm there were treatment-related reductions in body weight, which were more
6 pronounced in females. During the initial phase of the study, food consumption was reduced for
7 both sexes at 2000 ppm, although slightly increased food consumption was recorded for
8 females at this dose level during the second year of the study. There were no treatment related
9 clinical signs, ophthalmoscopic findings or effects on any haematological or urinalysis
10 parameters throughout the study. Slightly raised plasma alkaline phosphatase activity,
11 predominantly in females receiving 2000 ppm, and a slightly increased incidence of hepatocyte
12 fat and spongiosis hepatis in males at this dose level were considered as mild effects in the
13 liver.

14 The pathological examination showed no non-neoplastic and no neoplastic findings at any dose
15 level in either sex. There was an increased incidence of haemangiosarcoma in females receiving
16 2000 ppm, which was not statistically significant in the Fisher's Exact Test, but gave positive
17 results in the trend test. As this tumour type was not present in control females on this study,
18 and is relatively rare in the Alpk:APfSD strain of rat, an external peer review and a Pathology
19 Working Group (PWG) review was subsequently conducted to confirm the diagnosis of the
20 vascular neoplasms and to provide assistance in the interpretation of this equivocal result.
21 Following the PWG review and confirmed by a further independent expert, it was concluded
22 that the overall weight of evidence indicated that the slightly higher number of high dose
23 animals having vascular neoplasms of the liver is not associated with the dietary administration
24 of PHMB. RAC concluded that evidence from this rat study is not sufficient to conclude a clear
25 treatment-related effect with respect to vascular tumours.

26 Based on the findings of this study, a NOAEL of 2000 ppm PHMB in diet corresponding to 36
27 and 45 mg/kg bw/d was derived for male and female animals, respectively.
28

29 Ref.: 53, 13, 21, 30
30

31 Summary repeat-dose toxicity

32 Repeat-dose toxicity studies using different durations (3 weeks up to 2 years) and different
33 application routes (oral – drinking water; oral – diet; dermal; inhalation) have been performed
34 in different animal species (rat, mouse, Beagle dog).

35 Short-term and subchronic oral studies mainly served as range finder for studies of longer
36 duration. Subchronic dietary studies performed in rats and mice caused reductions in body
37 weight and effects on weight gain. In rats, the kidney was identified as target organ; there
38 were changes in renal function as well as changes in some plasma parameters (ALP, ALT, ASP).
39 However, interpretation of the dietary subchronic studies was hampered by limited
40 histopathologic examination.

41 In a dietary 12-month study in Beagle dogs, a NOAEL of 54 mg/kg bw was derived. At the
42 highest dose tested there were adverse effects on scrotal skin, indications of liver impairment
43 and histopathological findings in skin, liver and – for males only – in kidneys and testes. In a
44 dietary 2-year rat study NOAELs of 36 and 45 mg/kg bw/d were derived for male and female
45 animals, respectively. At the highest dose tested, most prominent findings were reductions in
46 body weight, changes in plasma parameters, the incidence of hepatocyte fat and spongiosis
47 hepatis. Further, the incidence of haemangiosarcomas was increased at the highest dose
48 tested.

1 From the latter study, the SCCS will take the NOAEL of 36 mg/kg bw/d for MoS calculation
2 /safety evaluation.
3
4
5

6 **3.3.6 Reproductive toxicity**

8 **3.3.6.1 Fertility and reproduction toxicity**

9
10
11 Two generation reproduction toxicity
12
13

14 Guideline: /
15 Species/strain: Rat/Alpk:APfSD (Wistar derived)
16 Group size: 26/sex/dose
17 Test substance: Vantocil P, 20.2% aqueous PHMB solution
18 Batch: D4097
19 Purity: 0.76 % impurities
20 Dose levels: 0, 200, 600, 2000 ppm
21 Route: Oral (diet)
22 Exposure period: Two successive generations including 10-week pre-mating period
23 GLP: Yes
24 Study period: 1993/1994, report dated 1995
25

26 Groups of animals received diets containing 0, 200, 600 and 2000 ppm Vantocol P
27 (corresponding to about 0, 23 – 24, 70 – 71, 239 – 249 mg/kg bw/day in males, 0, 25 – 26, 77
28 – 79, 258 – 270 mg/kg bw/day in females). After 10 weeks, animals were mated and allowed
29 to rear F1 litters to weaning. Selected F1 animals from F1 offspring were used as parental F1
30 animals for production of F2 offspring after an 11-week pre-mating period.

31 Parental animals and offspring were examined for mortality and clinical signs including unusual
32 behaviour. Body weight (all animals) and food consumption (F0 and F1 parental animals) were
33 determined at regular intervals. At termination, gross pathology was performed, weights of
34 kidney, liver, testes and epididymis were determined. Selected organs, grossly abnormal
35 tissues and reproductive organs were processed and examined by histopathology.

36 All pups surviving to termination and not selected for the next generation were killed on
37 approximately day 29 post-partum. Five F1 pups/sex and 10 F2 pups/sex received a full post
38 mortem examination.
39

40 Results

41 In adults receiving the highest dose, there was a decrease in body weight and decreased
42 efficiency of food utilisation during the pre-mating period, partly accompanied by reduced food
43 consumption. There was no evidence of an effect of PHMB on reproductive parameters or on
44 offspring growth and development up to the highest dose tested. There were some differences
45 in the weights of livers and kidneys in adults receiving 2000 ppm Vantocil P and in F2 offspring
46 at all dose levels. In the absence of related pathological changes these were considered not to
47 be biologically significant. There was no evidence of an effect of Vantocil P on any of the
48 reproductive parameters assessed. There was no evidence of an effect of PHMB on any of the

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1 pup parameters measured including growth and development. Based on decreased body
2 weights in parental animals, the authors derived a systemic, parental NOAEL of 600 ppm (70 –
3 79 mg/kg bw/d). The NOAEL for reproductive and offspring effects was 2000 ppm (239 – 270
4 mg/kg bw/d).

5
6
7
8 Ref.: 73

9 Other data on fertility and reproduction toxicity

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

21
22 From these litters a further 10 males and 20 females per group were selected to form the F1
23 parental generation. This process was repeated until the weaning of the F3 litter although half
24 of this litter was delivered by Caesarean section for the study of potential teratogenic effects.
25 Individual body weights, clinical condition and food consumption were recorded at regular
26 intervals. All pups delivered naturally were examined at birth and weaning and the number and
27 total weight per sex recorded. At weaning, 10 male and 10 females F3 pups were necropsied
28 and representative tissues stored for possible future histopathological examination.

29
30 Results

31 There were no indications of any adverse effect on body weight or food consumption in the F1
32 and P1 parental generations or on food consumption in the P2 generation. Evaluations of the
33 various reproductive indices, sex ratios, and body weight data of the fetuses taken by
34 Caesarean section and the offspring maintained through weaning revealed no meaningful
35 differences between the control and treated groups. Necropsy of weanlings did not reveal any
36 compound-related gross pathology. No findings indicative of embryotoxicity or teratogenicity
37 were noted in the fetuses taken by Caesarean section. The dose level of 1300 ppm
38 (corresponding to approximately 130 mg/kg bw/d) was concluded to be the NOAEL for general
39 toxicity, reproductive function and fertility as well as for pre- and postnatal development.

40 Ref.: 94
41
42
43

44 3.3.6.2 Developmental toxicity

45
46
47 Rats

48
49 Guideline: Comparable to OECD 414

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

12
13 Successfully mated female animals received diets containing test substance or aspirin from GD
14 1 until GD 20 when animals were killed. Body weight and food consumption were determined at
15 regular intervals. Dams were examined for mortality, clinical signs and abortion. Fetuses were
16 removed by Caesarian section, sexed, weighed and further investigated for external findings;
17 uteri were examined for resorptions. About one half of the fetuses of each litter were examined
18 for soft tissue findings and the remaining fetuses for skeletal findings.

19
20 Results

21 There were no mortalities and no adverse clinical effects in any group. Maternal weight gain
22 was significantly reduced in the groups receiving 1000 or 2000 ppm Baquacil SB and the food
23 consumption was also significantly reduced in these groups. Pregnancy was confirmed for 20 -
24 22 rats/group. Gestational parameters such as number of implantation sites, pre- and post-
25 implantation losses were not influenced by the test substance at any dose level. No dose-
26 related effects were observed on fetal or litter weights. There was an increase in extra ribs at
27 the high dose which was considered to be a consequence of maternal toxicity at this dose level.
28 There was no further substance-related effect on fetal morphology including ossification of the
29 skeleton in any of the treated groups. Based on reduced food consumption and body weight, a
30 maternal NOAEL of 200 ppm (corresponding to about 13 mg/kg bw/d) and a developmental
31 NOAEL of 1000 ppm (corresponding to about 54 mg/kg bw/d) were derived.

32 Ref.: 46
33
34

35 Mice

36
37 Guideline: Comparable to OECD 414
38 Species/strain: Mouse/Alderly Park strain
39 Group size: 47 – 49 mice in dosed groups; 25 control animals
40 Test substance: Baquacil SB (20 % aqueous PHMB solution)
41 Batch: no data
42 Dose levels: 0, 10, 20, 40 mg/kg bw/d (expressed as a.i.)
43 Vehicle: 0.5 % aqueous Tween 80
44 Exposure period: gestation days 6 – 15 (mating day taken as GD 0)
45 GLP: /
46 Study period: 1976

47
48 Results

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1 In dams at the top dose, the following effects were observed: increased mortality (6 dams),
2 reduced food intake (-23 % compared to controls between GD 8-14, $p < 0.01$) and reduced
3 bodyweight gain. Animals that died during the study were found to have macroscopic changes
4 in the stomach or caecum consistent with irritation and inflammation at site of contact. Signs of
5 recovery were evident post-dosing when this group showed increased food consumption and
6 final body weights similar to controls. In fetuses there was no effect of PHMB on the number,
7 growth or survival in utero except a slight increase in pre-implantation loss observed at 40
8 mg/kg (21.8 ± 25.6 vs 13.1 ± 15.2 in controls) and a significant increase in postimplantation
9 loss at 20 mg/kg (11.4 ± 19.7 % vs 6.1 ± 8.4 % in controls) attributed to an increase in early
10 intrauterine deaths. But the difference in preimplantation loss at 40 mg/kg was not statistically
11 significant and could not be related to PHMB as the dosing period began after implantation. The
12 post-implantation loss at 20 mg/kg was not seen at the highest dose and in the absence of
13 dose-response relationship, this effect was not attributed to treatment. There was no evidence
14 for teratogenicity. The percentage of fetuses with unossified 5th sternebrae or with fused 4th and
15 5th sternebrae was increased at the top dose, but in the absence of changes in fetal
16 development associated with PHMB, this finding was considered as unlikely to be substance-
17 related. A maternal NOAEL of 20 mg/kg bw/d and a developmental NOAEL of 40 mg/kg bw/d
18 were derived from this study.

19 Ref.: 9, 7, 8
20

21 In an older, non GLP- and non-Guideline compliant teratology study, test material IL 780,
22 SDGM 5642 was investigated in groups of 14 – 15 mated female White New Zealand rabbits at
23 dose levels of 0, 10, 40 and 160 mg/kg bw/d after oral (gavage) administration. The
24 administration of 10 and 40 mg/kg bw/day had no effect on pregnancy or on maternal and fetal
25 survival. The number of soft tissue or skeletal abnormalities did not differ from the number in
26 the control group at these dose levels. At 160 mg/kg bw/day maternal toxicity was reported
27 which led to impaired implantation, increased early fetotoxicity and increased resorbed and
28 dead fetuses.

29 Ref.: 3
30

31 Conclusions on reproductive toxicity

32 In an oral chronic toxicity study in Beagle dogs, histopathologic changes in testes were
33 observed at the highest dose tested (169 mg/kg bw/d, reduced to 108 mg/kg bw/d). In a 2-
34 and a 3-generation reproductive toxicity study in rats, no adverse effects on the reproductive
35 parameters addressed were observed. No evidence of fetal/developmental toxicity independent
36 from maternal toxicity was observed in developmental toxicity studies in rats and rabbits.
37 There were indications for retarded ossification at 20 and 40 mg/kg bw/d in a developmental
38 toxicity study in mice, which was not considered an adverse effect by the SCCS.
39
40
41

42 **3.3.7 Mutagenicity / Genotoxicity**

43 44 45 3.3.7.1 Mutagenicity / genotoxicity *in vitro*

46 Bacterial Reverse Mutation Test
47
48

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1	Guideline:	OECD TG 471
2	Test system:	Salmonella typhimurium strains TA98, TA100, TA1535, TA1537,
3		TA1538
4	Replicates:	Triplicate plates
5	Test substance:	Vantocil IB, 19.6 % aqueous solution
6	Batch:	Bx2125
7	Solvent:	DMSO
8	Concentrations:	Range-finding (TA100 only):
9		±S9-mix: 0, 1.6, 8.0, 40, 200, 1000, 5000 µg/plate
10		Experiment I + II:
11		±S9-mix: 0, 0.32, 1.6, 8.0, 40, 200, 500 µg/plate
12	Treatment:	plate incorporation assay with minor modifications
13	GLP:	yes
14	Study period:	1988, report dated 1989

15
16 PHMB was investigated in the bacterial reverse mutation test with and without metabolic
17 activation by S9-mix prepared from Aroclor 1254 induced liver from Alderley Park rats. In a
18 range-finding experiment, significant toxicity was observed at concentrations > 200 µg/plate in
19 TA100 ± S9. Therefore, 500 µg/plate was used as the top concentration in the 2 main
20 experiments. For control purposes, the negative, vehicle (DMSO) and positive controls (+ S9:
21 N-methyl-N-nitro-N-nitrosoguanidine (MNNG) for TA 100 and TA 1535, Daunorubicin (DR) for
22 TA98, Acridine mutagen ICR191 for TA1537 and 4-nitro-O-phenylenediamine (4- NPD) for TA
23 1538; -S9: 2-aminoanthracene (2-AA) for all strains) were also investigated.

24
25 Results:

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

33 Ref.: 14

34
35 An earlier bacterial reverse mutation test which preceded OECD test guidelines and GLP has
36 been performed with Vantocil IB (20 % aqueous solution), batch AGDM 2253/77, in *Salmonella*
37 *typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 ± S9 mix. PHMB was toxic at a
38 concentration of 333.3 mg per plate, particularly in strains TA98, TA100, and TA1535, and was
39 shown to be weakly mutagenic in strain TA1538 in the absence of metabolic activation.

40 Ref.: 43

41
42 A further bacterial reverse mutation test performed in compliance with GLP also investigated
43 Vantocil IB (20 % aqueous solution), batch AGDM 2253/77, in *Salmonella typhimurium* strains
44 TA98, TA100, TA1535, TA1537, TA1538 ± S9 mix from livers of Aroclor 1254 induced Sprague-
45 Dawley rats. Test substance concentrations were up to 500 µg/plate.

46 Vantocil IB gave a negative response with all strains ± S9 mix. All tester strains were sensitive
47 to the appropriate positive controls (N-2-fluorenylacetamide for TA98 and TA1538; 1,3-
48 propanesultone for TA100 and TA 1535 and 9-aminoacridine for TA1537).

49 Ref. 92

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1
2 **SCCS comment**

3 In an earlier bacterial reverse mutation test, Vantocil P was toxic in several bacterial strains
4 and showed to be mutagenic. As Vantocil P is a bactericide, the SCCS considers the Ames test
5 as not suitable for mutagenicity testing. This is also stated in a revised version of the Notes of
6 Guidance.
7
8
9

10 *In vitro* mammalian cell gene mutation test

11
12 Guideline: OECD TG 476
13 Species/strain: L5178Y TK+/- Mouse Lymphoma cells
14 Replicates: 2
15 Test substance: PHMB P20D
16 Vehicle: culture medium
17 Batch: 1202
18 Purity: /
19 Concentrations: -S9 mix: 0, 12.5, 25, 50 and 100 µg/ml
20 +S9 mix: 0, 12.5, 25, 50 and 100 µg/ml
21 Positive control: -S9 mix: methyl methanesulfonate (MMS, 2 and 10 µg/ml)
22 Positive Control: +S9-mix: cyclophosphamide (2 µg/ml)
23 Negative control: solvent control
24 Treatment: 3 h and 20 h
25 GLP: yes
26 Study period: 2002
27
28

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

35 Results: at 50 and 100 µg/ml, cytotoxicity of PHMB was higher than that of the positive
36 controls. No significant increases in mutant frequencies were observed for PHMB in two
37 independent assays at any concentration tested in the presence or absence of S9-mix.

38 Analysis of the size of colonies did not show any change in small and large colony number at
39 any concentration tested in the presence or absence of S9-mix. MMS on the other hand
40 produced clear increases in mutation frequencies in the absence of S9-mix and
41 cyclophosphamide produced significant increases in mutation frequencies in the presence of
42 S9-mix. Both positive controls produced a clear increase in small colony levels. Thus, positive
43 and negative controls confirm the validity of the assay.

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

46 Ref.: Harmand (2002)
47
48
49

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 **SCCS comment**

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

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

13
14
15 Guideline: similar to OECD TG 476
16 Species/strain: Mouse lymphoma cell line P388 (tk+/-)
17 Replicates: 5 replicate cultures
18 Test substance: Vantocil P (20% aqueous solution)
19 Vehicle: DMSO
20 Batch: ADGM/1021/79
21 Purity: /
22 Concentrations: -S9 mix: 25, 250, 500, 1000, 2000 µg/ml
23 +S9 mix: 25, 250, 1000, 2000 µg/ml
24 Positive control: ±S9 mix: methyl methanesulfonate (MMS), 32.5 and 65 µg/ml
25 Negative control: DMSO
26 Treatment: 30 minutes
27 GLP: yes
28 Study period: 1980 – 1981
29
30

31 PHMB was diluted with DMSO and added to the cell cultures for 30 minutes at indicated
32 concentration levels and the cell suspensions in the test tubes were rotated on a flat-bed test
33 tube roller. S9 was obtained from livers of male Sprague-Dawley rats treated with Aroclor
34 1254. After exposure, the cells were washed and maintained in medium for an expression time
35 between 68 – 72 hr at 37° C. Thereafter, 5-iodo-2'-deoxyuridine (IUdR) as selective agent and
36 molten agar were added to the test tubes. The mixtures were shaken, dispensed into petri
37 dishes and placed in a CO2 incubator until colony formation. The colonies were counted and the
38 mean of 5 replicates was determined. Positive and negative controls were tested in parallel.

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

45 The study authors concluded that PHMB was not mutagenic as it did not induce gene mutations
46 at the tk locus in P388 mouse lymphoma cells in the absence and presence of metabolic
47 activation up to cytotoxic concentrations.
48
49

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 **SCCS comment**

2 Exposure time is lower than recommended in OECD TG 476. Additionally, only short time
3 exposure was used and no longer exposure as recommended by the recently revised guideline.
4 The cell line used does not belong to those recommended in OECD TG 476.
5 Therefore, the results have limited value.
6
7

8 ***In vitro* Micronucleus Test in human lymphocytes**
9

10 Guideline: OECD TG 473
11 Species/strain: Cultured human peripheral blood lymphocytes from two volunteers
12 (male and female)
13 Replicates: Duplicate cultures, two independent experiments
14 Test substance: Vantocil IB (19.6% aqueous PHMB solution)
15 Batch: Bx2125
16 Concentrations: -S9 mix: 0, 5.0, 25, 50 µg/mL (both volunteers)+S9 mix: 0, 25, 100,
17 187.5 µg/mL (volunteer 1)
18 +S9 mix: 0, 25, 100, 250 µg/mL (volunteer 2)
19 Solvent/negative
20 control: 0.85% physiological saline
21 Positive Controls: -S9 mix: Mitomycin C (MMC), 0.5 µg/mL
22 +S9 mix: Cyclophosphamide (CPA), 100 µg/mL
23 GLP: Yes
24 Study period: 1989
25

26 The potential of PHMB to induce chromosome aberrations was investigated in cultured human
27 peripheral blood lymphocytes from two volunteers in the presence or absence of S9 (from
28 livers of male Alpk:APfSD rats induced with Aroclor 1254). PHMB was administered to the
29 cultures approximately 44 hr after initiation and remained in contact with the cells for 2 hr 20
30 minutes to 3 hr 40 minutes. 70 hr after culture initiation, dividing lymphocytes were arrested in
31 metaphase by treatment with 10 µg/ml colchicine and 2 hr later the cells were subjected to
32 hypotonic treatment for 12 minutes in 0.075M KCl followed by fixation in glacial acetic
33 acid/methanol and staining in Giemsa. The appropriate concentration levels selected for
34 chromosomal analysis were 50, 25 and 5 µg/ml for both volunteers -S9 and 250, 100 and 25
35 µg/ml for volunteer 2 +S9 and 187.5, 100 and 25 µg/ml for volunteer 1 + S9.
36

37 **Results**

38 The overall mitotic activity was reduced by approximately 50 - 80 %. In any case, PHMB could
39 be examined for clastogenicity at concentration levels up to and including those causing clear
40 cytotoxic effects. No statistically or biologically significant increases in chromosomal damage
41 were seen in any cultures treated with PHMB. The sensitivity and validity of the test system
42 used was demonstrated as positive controls induced a significant increase in cells with
43 structural aberrations.

44 The study authors concluded that PHMB did not induce chromosomal aberrations in cultured
45 human lymphocytes under the test conditions applied.
46

Ref.: 54
47
48
49

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 **SCCS comments**

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

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

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

15 Ref.: 84

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

22 3.3.7.2 Mutagenicity / genotoxicity *in vivo*

24 ***In vivo* Mammalian Bone Marrow Micronucleus Test**

25 Guideline: /
26 Species/strain: Mouse, C57BL/6JfCD-1/Alpk
27 Group size: 5 males, 5 females per group
28 Test substance: Vantocil IB (19.6 % aqueous PHMB solution)
29 Batch: BX 2125 Ex. Grangemouth
30 Purity: 100 %
31 Dose levels: 0, 250, 400 mg/kg bw (no statement on correction for concentration)
32 Exposure: Single application
33 Route: Oral (gavage)
34 Vehicle: Double deionised water
35 Positive control: Cyclophosphamide (CPA, 65 mg/kg bw)
36 GLP: yes
37 Study period: 1988 – 1989

38
39 Dose levels were selected based on the findings of oral dosing of 500 and 2500 mg/kg bw
40 followed by a 4-day observation period. Test substance was applied at dose levels of 0, 250
41 and 400 mg/kg bw. Animals were killed at 24, 48 and 72 hr after dosing (animals treated with
42 positive control were killed at 24 hr only) and bone marrow was obtained, processed, and
43 stained. For each animal, initially 1000 polychromatic erythrocytes were scored for the
44 presence of micronuclei. The portion of polychromatic erythrocytes to total erythrocytes was
45 recorded per 1000 erythrocytes.

46
47 **Results**

48 No mortality occurred in any male or female mouse at any dose level. The high dose of 400
49 mg/kg bw/day led to a reduction in polychromatic erythrocytes as an indication of availability of

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 substance in the bone marrow. No statistically significant increase in the frequency of
2 Micronucleated polychromatic erythrocytes (MPE) was observed when the sexes were
3 combined. However, when the sexes were considered separately, a small statistically significant
4 increase in MPE was apparent in male mice at the 400 mg/kg bw/day dose level at the 48 hr
5 sampling time. Therefore, a further 2000 PCE were examined for the male 400 mg/kg bw/day
6 group and the male negative control group at all sampling times.
7 Following the extended counts, there was no difference between the test and control male
8 animals at the 48 hr sampling time and no statistically significant differences were obtained
9 whether the extended counts were analysed alone (as 2000 PCE) or combined with the original
10 counts (as 3000 PCE).
11 The positive control caused chromosomal aberrations.
12 Based on these findings, the study authors concluded that under the conditions applied,
13 Vantocil IB is not clastogenic *in vivo*.

Ref.: 83

17 ***In vivo* Unscheduled DNA synthesis**

18 Guideline: mainly comparable to OECD 486
19 Species/strain: Rat/Alpk:APfSD (Wistar derived)
20 Replicates: Two independent experiments
21 Group size: 2 - 3 male animals per dose and experiment
22 Test substance: Vantocil IB, 19.6 % aqueous PHMB solution
23 Batch: BX 2125
24 Purity: /
25 Dose levels: 0, 750, 1500 mg/kg bw (no correction for concentration or purity)
26 Exposure: Single application
27 Route: Oral (gavage)
28 Vehicle: for test substance: Double deionised water
29 Positive control: corn oil
30 Positive control: 6-p-dimethylaminophenyylazobenzthiazole (6-BT, 40 mg/kg bw)
31 Exposure period: 4, 12 h after application
32 GLP: yes
33 Study period: 1989

35 Dose levels were selected based on ref. 55. Vantocil IB was administered by gavage at dose
36 levels of 375 (not evaluated), 750 and 1500 mg/kg bw (application volume: 10 ml/kg bw) for a
37 period of 4 or 12 hr to groups of 2 - 3 male rats per dose and experiment. Two independent
38 experiments were carried out. The application volume was 10 ml/kg bw. After the respective
39 treatment period, animals were sacrificed, liver perfusion was carried out and primary
40 hepatocytes cultures were established from each animal. Hepatocyte cultures were exposed for
41 4 hr to [3H]-thymidine, slides were prepared and net nuclear grain counts were determined by
42 counting 100 cells per animal.

44 **Results**

45 Overt clinical findings in the form of excessive salivation and subdued attitude were observed
46 at 1500 mg/kg bw. Treatment with 750 or 1500 mg/kg bw Vantocil IB revealed no UDS
47 induction in the hepatocytes of the treated animals as compared to the corresponding vehicle
48 controls. The positive control produced the expected response.

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

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

3 Ref.: 93
4
5

6 Genotoxicity data on monomers from which PHMB has been synthesised are:

- 7 • Hexamethylenediamine (HMD): CAS 124-09-4

8 OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test), result negative

9 OECD 471 (*in vitro* gene mutation study in bacteria), result negative

10 Ref.: 122

- 11 • Hexamethylenebisdicyandiamide (HMBDA): EC 240-032-4, CAS 15894-70-9

12 OECD 476, *In vitro* Mammalian Cell Gene Mutation Test, result negative

13
14 Ref.: 123

15 **SCCS conclusion on Mutagenicity/Genotoxicity**

16 The bacterial reverse mutation test is considered not suitable to be used as PHMB is a
17 bactericide. PHMB was negative in a well-performed gene mutation assay in mammalian cells
18 and in an *in vitro* chromosome aberration test in human lymphocytes. An *in vitro* micronucleus
19 test was also negative but only short treatment times were used. The negative results found in
20 *in vitro* tests were confirmed under *in vivo* conditions as PHMB did not lead to the induction of
21 micronucleated polychromatic erythrocytes in mice. These negative results in the *in vivo*
22 micronucleus test were further supported by a negative *in vivo* UDS test.

23 Consequently, based on the available data, PHMB can be considered to have no genotoxic
24 potential and additional tests are unnecessary.
25
26

27 **3.3.8 Carcinogenicity**

28
29
30 ORAL

31
32 Mouse

33
34 Guideline: US EPA 83-2 (comparable to OECD TG 453)

35 Species/strain: Mouse, C57Bl/10J/CD-1 Alpk

36 Group size: 55/sex/dose

37 Test substance: Vantocil P (20.2% aqueous PHMB solution)

38 Batch: D4097

39 Purity: impurities < 0.76 %

40 Vehicle:

41 Dose levels: 0, 400, 1200 and 4000 ppm (correction for 20.2 % PHMB content)

42 Route: oral

43 Administration: diet, 2 years

44 GLP: yes

45 Study period: 1992 – 1994, report dated 1996
46

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1 Dietary levels of PHMB corresponded to about 0, 55, 167, 715 mg/kg bw/day in males and 0,
2 69, 217, 856 mg/kg bw/day in females. The animals were examined for mortality and clinical
3 signs including unusual behaviour. Body weight and food consumption were determined at
4 regular intervals. Blood samples for haematology were collected from all animals at scheduled
5 termination and bone marrow smears were taken from all animals and examined for females in
6 the control and high dose groups only. All animals surviving to termination were anaesthetised,
7 killed by exsanguination and subjected to a complete necropsy. Testes, kidneys, adrenal
8 glands, liver and brain were weighed. A comprehensive number of organs and tissues were
9 processed, fixed, stained and examined by histopathology.

10
11
12 Results

13
14 Non-neoplastic findings

15 4000 ppm was considered as a dose exceeding the MTD. Body weights were reduced up to 20
16 % (males) and 15 % (females) compared to controls in the second year of the study.

17 Compared to controls, the reduction of body weight gain was 35-42 % (males) and 22-33 %
18 (females) during weeks 53- 79 of the study. Food consumption and mortalities were increased,
19 food utilisation was decreased. Haemangiosarcoma was the most frequent factor causing
20 death. The main treatment related clinical observation in males and females at the 4000 ppm
21 was anal swelling. The first noted occurrence was in week 18 for males and week 53 for
22 females. At termination, there was an increase in haemoglobin, haematocrit and red cell count
23 in both sexes receiving 4000 ppm. Various non-neoplastic changes were seen in the liver, gall
24 bladder and recto-anal junction.

25 1200 ppm was considered as MTD. Compared to controls, body weights were 5 -6 % lower
26 during the 2nd year of the study and the reduction of body weight gain was 7 - 14 % (males)
27 and 5 - 10 % (females) during weeks 53- 79 of the study. There were non-neoplastic findings
28 of inflammatory nature in the recto-anal junction and non-neoplastic changes in livers (both
29 sexes) and gall bladder (females only). In females, haemoglobin, haematocrit and red cell
30 count were increased. There was also an increase in the number of mice with torn ears and hair
31 loss at 400 and 1200 ppm. At 400 ppm, non-neoplastic findings consisted mainly in minimal
32 inflammation of ano-rectal junctions.

33
34 Neoplastic findings

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

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

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

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1 control incidences. In males at 1200 ppm, the increase of haemangiosarcomas in the liver was
2 considered small.

3
4 Ref.: 74

5 The study was discussed by several expert groups.

6 As the altered tumor profile was present at a dose exceeding the MTD and as
7 haemangiosarcoma is a common tumor in C57 mice, the findings of the study were first
8 reevaluated by a pathology working group. Although no statistical analyses were presented, the
9 incidences of haemangiomas and of haemangiosarcomas (and combined incidences thereof) in
10 the liver and any sites of high dose male and female mice were considered to be clearly above
11 control incidences. In males at 1200 ppm, the increase of haemangiosarcomas in the liver was
12 considered a chance event. After statistical analysis of the same data, it was shown that when
13 the MTD exceeding top-dose (4000 ppm) was excluded from any analyses, no statistically
14 significant results were observed in either males or females for haemangioma,
15 haemangiosarcomas or total incidence of vascular tumors combined.

16 However, at 4000 ppm the incidence was increased in males and females but with statistically
17 significance only in males. More recently, RAC stated that it would be unlikely to explain higher
18 rates of vascular tumour in the liver by chance and that this study gives some evidence of
19 carcinogenicity.

20 Ref.: 70, 37, 30
21
22

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

29 All offspring were weaned at 3 weeks of age. At 5 weeks of age, 50 males and 50 females were
30 selected from each group. The offspring were fed the same diets as the parents throughout the
31 experiment. After a further 80 weeks, 10 males and 10 females per group were killed for
32 pathological examination. The experiment was terminated when the overall mortality had
33 reached 80 %, 97 weeks after selection of offspring.

34 Mortality was high due to high fighting of the males. Mean body weight gain in females was
35 dose-dependently decreased and absolute liver weights in males and females were increased at
36 1000 ppm (significant for females only). There were no treatment-related (non-neoplastic or
37 neoplastic) increases in histopathologic findings. However, with respect to vascular tumours of
38 concern, there were some treated animals bearing haemangiomas or haemangiosarcomas in
39 the liver or at other sites.

40 Ref.: 18
41
42

43 Rat

44 A combined chronic toxicity/carcinogenicity study performed in rats is described in section
45 3.3.5.3.

46 There exists another, older non-GLP and non-guideline compliant long-term feeding study in
47 rats which is of questionable reliability due to infections and less than 50 % survival at the end
48 of study. Groups of 60 male and 60 female rats of an unspecified strain were fed PHMB
49 (Baquacil SB, batches: SDC/596 and ADGM 5911, 20 % aqueous PHMB solution) at dose levels

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1 of 0, 200, 1000 and 2000 ppm. The study was terminated at 124 weeks, when 80 % mortality
2 occurred. There were 2 outbreaks of infection. The respiratory infection at week 70 and week
3 103 caused a rise in the mortality rates. No observable clinical effects were produced by the
4 compound and apart from a slight anaemia in the top dose at 104 weeks, no adverse changes
5 were seen. The administration of Baquacil SB caused some growth depression in treated
6 animals. Long-term exposure to PHMB was not related to toxic or carcinogenic effects.
7 Haemangiomas were found at interim kills at week 52 in 1/12 male rats (mesenteric lymph
8 nodes) at 200 ppm and 1/12 male rats at 200 ppm (cervical lymph nodes), at interim kill at
9 104 weeks in 2/12 males at 1000 ppm (mesenteric lymph nodes) and in 1/8 females at 200
10 ppm (uterus) as well as at the end of study at week 124 in 1/20 males at 1000 ppm
11 (mesenteric lymph nodes), 1/19 males at 2000 ppm (spleen) and one haemangiosarcoma at
12 week 104 in 1/21 males at 2000 ppm (mesenteric lymph nodes). No vascular tumours were
13 seen in controls.

14 Ref.: 4

15
16
17

18 DERMAL

19

20 Guideline: Precedes guideline programmes
21 Species/strain: Mouse, Alpk:APfCD-1
22 Group size: 50/sex/dose
23 Test substance: Baquacil SB, (20.0 % aqueous PHMB solution)
24 Batch: S DC/596, ADGM 5911
25 Purity:
26 Vehicle: ethanol
27 Dose levels: 0, 0.6, 6.0, 30 mg/mouse/day (corresponding to about 0, 15, 150, 750
28 mg/kg bw/day)
29 Dose volume :
30 Route: dermal, non-occluded
31 Administration: daily, 80 weeks
32 GLP: precedes GLP
33 Study period: report dated 1977

34

35 Aqueous PHMB formulated in ethanol was applied to the shorn backs of groups of mice at dose
36 levels of 0, 0.6, 6.0 or 30 mg/mouse/day (corresponding to approximately 0, 15, 150 or 750
37 mg/kg bw/day), 5 days per week for 80 weeks. Clinical observations (including
38 ophthalmoscopy), bodyweights and food consumption were recorded. All animals were
39 subjected to a post-mortem examination. A full range of tissues were taken for
40 histopathological examination.

41

42

43 Results

44 The highest dose of 30 mg/mouse/day was considered as exceeding the MTD and led to irritant
45 effects on the skin and to a general poor condition, which was accompanied by body weight
46 loss and a high incidence in mortality (76-78 % of animals dying prior to study termination).
47 There was only a transitory skin irritant effect on male mice receiving 6.0 mg/mouse/day and
48 some reduction in body weight gain. There was no effect at 0.6 mg/mouse/day. There was a

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1 variety of inflammatory hepatic changes in all groups including controls, but at 30
2 mg/mouse/day this was characterised by a severe hepatitis in some animals.
3 There was a slight increase in the incidence of liver tumors observed at 30 mg/mouse/day (four
4 in the control and ten at 30 mg/mouse/day). This was statistically significant only in the case of
5 liver tumors of endothelial origin (both benign and malignant; two in the control and six at 30
6 mg/mouse/day), when the data were pooled over the whole study period and both sexes.
7 There was no evidence of such an effect in animals receiving 0.6 or 6.0 mg/mouse/day. An
8 overall NOAEL of 0.6 mg/mouse/day corresponding to 15 mg/kg bw/d was derived from this
9 study.

10 Ref.: 17

11
12 **SCCS conclusion on carcinogenicity**

13 In a rat oral chronic toxicity/carcinogenicity assay, an increased incidence of
14 haemangiosarcomas (statistically not significant) was observed at the highest dose of 2000
15 ppm PHMB. Recently it has been concluded (Ref. 30) that evidence from the chronic rat study
16 is not sufficient to demonstrate a clear treatment-related effect. In an oral study in mice, PHMB
17 increased the incidence of vascular tumors, mainly in the liver. In a dermal study in mice, a
18 statistically significant increase in the incidence of haemangiosarcomas was observed in
19 females at the highest dose (750 mg/kg bw/d; considered to exceed MTD) tested. Based on the
20 outcome of the above studies a harmonized classification as Carc 2 H351 (suspected of causing
21 cancer) has been assigned according to Annex VI of Regulation (EC) No. 1272/2008.
22
23

24 **3.3.9 Photo-induced toxicity**

25
26 **3.3.9.1 Phototoxicity / photo-irritation and photosensitisation**

27
28
29 The purpose of the study was to determine the safety of the test material following repeated
30 applications to human panellists and exposure of the sites to natural Florida sunlight.
31

32 Guideline: Adaptation of the repeat insult patch test (RIPT) procedure of Draize
33 Species: Human
34 Group size: 26 volunteers (13 males, 13 females, aged <21 - >60 years)
35 Test substance: Baquacil SB (20 % aqueous PHMB solution)
36 Batch: ADGM 3429 (4/2/75)
37 Vehicle: Distilled water; 0.01 % SLS
38 Route: occlusive epicutaneous application on the upper arm
39 Induction: three times/week for 3 or 4 weeks for a total of 9 – 12 applications
40 Challenge: 6 weeks after initial application
41 Exposure duration: 24 h
42 Concentrations: 1 % PHMB
43 Light source: Direct rays of mid-day sun
44 Light exposure: immediately after patch removal, 1 hr
45 Skin readings: during induction: prior to 2nd through 9th application and on Monday
46 following 9th application 48, 96 hr after application
47 after challenge: 48, 96 hr after application
48 GLP: not stated

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1 Study period: 1976
2

3 Volunteers received patch applications of 20 x 20 mm patches of 0.4 ml of an aqueous solution
4 of Baquacil SB corresponding to 1 % a.i., which had been supplemented by SLS (final
5 concentration of SLS: 0.01 %) in order to increase dermal penetration. Patches were applied to
6 the upper arms of the panellists three times per week for 3 or 4 successive weeks and removed
7 24 hr after each application. Immediately after patch removal, test sites were exposed for one
8 hr to mid-day sun rays (panellists of the 3-week treatment started sun exposure in week 2
9 only). Reactions to all patches were scored before starting the second and the subsequent
10 applications (until the 9th application).

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

14
15 **Results**

16 During the induction phase, erythema and oedema were observed at certain occasions in 5
17 panelists. After challenge exposure, no reactions were observed.
18
19

20 **SCCS comment**

21 The SCCS does not consider HRIPT studies for determining sensitisation potential to be ethical.
22 From the results it can be concluded that Baquacil SB had some irritating but no
23 photosensitising potential.

24 Ref.: 44
25

26 **SCCS conclusion on phototoxicity**

27 One non-standardised photo-sensitising test is available. No studies on photomutagenicity or
28 photoclastogenicity have been performed. Based on the available data, PHMB cannot be
29 considered as a photosensitising or phototoxic substance.
30
31

32 3.3.9.2 Phototoxicity / photomutagenicity / photoclastogenicity
33

34 /
35
36
37

38 **3.3.10 Human data**
39

40
41 **HRIPT Tests**

42
43 **Guideline:** Insult patch test according to standards set by Japanese patch test study
44 group.
45 **Species:** Human
46 **Group size:** 45 volunteers (17 males, 28 females)
47 **Test substance:** Cosmocil CQ (20 % aqueous PHMB solution)
48 **Batch:** 0237

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1 Vehicle: Purified water
2 Concentrations: 0.3, 0.6 and 1.5 % a.i.
3 Route: Topical application to the medial surface of the upper arm
4 Exposure duration: 24 h
5 GLP: Yes
6 Study period: 2001
7

8 Finn-chamber plasters dosed with the indicated test concentrations were applied to the skin of
9 the medial part of the arm. The plasters were removed after 24 hr and skin reactions were
10 examined 30 min after exposure (24-hr reading) and 24 hr thereafter (48-hr reading).
11 Skin reactions were scored according to the "Standards set by the Japanese Patch Test Study
12 Group."
13

14 **Results**

15 Plaster dermatitis was observed in all test groups, including vehicle controls. Only the highest
16 test material concentration caused skin reactions at both time points, whereas lower
17 concentrations and vehicle produced only reactions at the 24 hr time point. Skin irritation
18 indices of 6.6, 5.5, 5.5 and 8.8 were obtained for concentrations of 0 (vehicle control), 0.3, 0.6
19 and 1.5 % a.i. The study authors considered the test material free of undesirable primary skin
20 irritation property as skin irritation indices of test material solutions were almost the same as
21 the irritation index of vehicle control.
22

Ref.: 91

23
24 **SCCS comment**

25 The test report was the English translation of a Japanese test report. The SCCS does not
26 consider HRIPT studies for determining sensitisation potential to be ethical.
27 As the contribution of plaster dermatitis cannot be clearly discerned from test-article induced
28 dermatitis, no clear conclusions can be drawn from this study. It can be assumed that the test
29 material is slightly irritating under the conditions of this test.
30
31
32

33 Guideline: HRIPT according to the method of Shelanski, 1951, Shelanski and
34 Shelanski, 1953 and Stotts, 1980*)
35 Species: Human
36 Group size: 191 of 209 volunteers completed
37 49 in panel 1, 114 in panel 2, 28 in panel 3
38 Test substance: Vantocil IB (20 % aqueous PHBM solution)
39 Batch: Code Y 00156/001/005
40 Vehicle: distilled water
41 Concentrations: for induction: 2 % and 4 % a.i.
42 For challenge: 0.05, 0.1, 0.2, 0.5, 1 and 2 % a.i.
43 Route: dermal (topical application to the dorsal surface of the upper arm)
44 Exposure duration: 24 hr
45 GLP: not stated
46 Study period: 1981
47

48 For induction, 2 x 2 cm patches moistened with 0.5 ml aliquots of sample dilutions were applied
49 to the dorsal surface of the upper arms for 24 hr. Induction patches were applied 3 times per

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1 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 in 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 sensitiser in humans in concentrations of at least 2 %.

Clinical patch tests, case reports and medical surveillance data

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1 The working group of Schnuch et al. conducted patch tests on a total of 1554 male and female
2 patients suspected to have contact allergies to medications and cosmetics in 1998, in
3 accordance to recommendations of the International Contact Dermatitis Research Group
4 (ICDRG) and the German Contact Dermatitis Research Group (DKG). The patients were
5 exposed to PHMB at 2.5 % in an aqueous solution. 389 patients were exposed for 1 day and
6 1165 for 2 days. The skin reactions were scored on day 3. Six patients (0.4 %) showed a
7 positive skin reaction. One of the reactions in a patient with atopic dermatitis may have been
8 false positive. The authors concluded that PHMB sensitisation can be considered to be
9 extremely rare.

10 Ref. 86

11
12 The same working group performed a follow-up study. PHMB received as Cosmocil CQ was
13 prepared for patch testing at concentrations of 2.5 % and 5 % PHMB in water. The
14 preparations were tested in parallel in 1975 unselected patients from 1 July to 31 December
15 2005. Frequencies of sensitisation (as % of patients tested) were calculated as crude
16 proportions and additionally standardised for sex and age. Ten patients (0.5 %) showed a
17 positive skin reaction to PHMB at 2.5 % and 16 patients (0.8 %) to PHMB at 5 %. However, the
18 authors assumed that probably at least 4 reactions at 2.5 % may be doubtful or irritant, i.e.
19 false positive, as they were not confirmed by simultaneous reactions to higher concentrations.
20 Potential causal exposures were assessed by a case-by-case analysis and by referring to
21 surrogate markers of exposure in terms of concomitant reactions. Occupational exposures were
22 identified as a probable cause of sensitisation. Further risk factors included leg dermatitis and
23 old age. In agreement with the previous study, the frequency of sensitisation remained very
24 low. The authors finally concluded that it is very unlikely that exposure to cosmetics or
25 personal care products may have played a role in the few cases where people were sensitised.

26 Ref.: 87

27
28 In agreement to the above studies, in an earlier study (apparently a meeting abstract which
29 was not available to the SCCS), 2 out of 374 patients (0.5 %) reacted positively to PHMB patch
30 tested at 2.5 % PHMB in water. (McFadden, 1998, cited in Ref. 30 and 86).
31 In addition, there are sporadic case reports on sensitisation to PHMB, such as, for example,
32 from wet wipes.

33 Ref.: SCCS Ref. VI

34
35 (ref: Leysen J, Goossens A, Lambert J, Aerts O (2014) Polyhexamethylene biguanide is a
36 relevant skin sensitiser in wet wipes. Contact Dermatitis 70:323-325 Based on medical
37 surveillance information obtained between 2004 and 2007 from employees working with PHMB,
38 no case of skin sensitisation due to PHMB was reported. All manufacturing and laboratory
39 employees were offered complete medical evaluations on a regular basis depending on their
40 age. These were conducted every one to two years. In addition, employees who work with skin
41 sensitisers participated in the "Skin Sensitizer Medical Surveillance Program". These employees
42 were examined every six months for signs of skin sensitisation.

43 Ref.: 38

44
45 **SCCS conclusions on human data**

46 From HRIPT tests it can be concluded that a concentration of 2 % PHMB is capable of causing
47 skin sensitisation in humans which can be elicited at concentrations starting from 0.2 % a.i.
48 From reports on patch tests, PHMB sensitisation can be considered of low frequency (up to 0.5
49 % in dermatitis patients).

3.3.11 Special investigations

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 and did not activate macrophages and the 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

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1 aspartate aminotransferase in plasma obtained at sacrifice. Plasma endotoxin levels were
2 quantified using an endotoxin assay kit. Oxidative stress was measured by detection of 8-
3 Hydroxydeoxyguanosine (OH8dG) in isolated DNA from livers. PHMB did not induce
4 hepatotoxicity at any concentration or time point. At 4000 ppm body weight was transiently
5 decreased and there was a thinning of the stomach wall but at 28 days of exposure, no effect
6 on body weight or liver weight was observed at any dose. PHMB increased cell proliferation in a
7 dose-related manner at 1200 and 4000 ppm. Cell proliferation was also increased at 1200 ppm
8 PHMB following 14 days exposure. PHMB increased plasmaendotoxin, a known activator of
9 macrophages, at 1200 and 4000 ppm for 28 days and at 100 and 200 ppm for 14 days, but not
10 for longer duration.

11
12 *In vitro* and *in vivo* results point to an indirect effect of PHMB on liver endothelial cells. The
13 increase in endothelial cell growth occurred with a threshold at 400 ppm and was dose related.
14 Endotoxin-mediated activation of macrophages might be involved, but other mechanisms
15 cannot be excluded.

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Ref.: 64

In vitro Mammalian cell transformation assay

22 Guideline: Mammalian cell transformation assay according to Styles, 1977
23 Test system: Baby hamster kidney fibroblasts (BHK21/C13)
24 Replicates: quadruplicate plates, two independent experiments
25 Test substance: Vantocil IB (19.6% aqueous PHMB solution)
26 Batch: ORG/42/78, ADGM 2253/77
27 Concentrations: Experiment I: +S9 mix: 0, 0.25, 2.5, 25, 250, 2500 µg/mL
28 Experiment II: +S9 mix: 0, 25, 250, 750, 1500, 3000 µg/mL
29 Vehicle: DMSO
30 Positive Controls: Experiment I: Benzidine: 0.25, 2.5, 25, 250, 2500 µg/mL
31 Experiment II: Acrylonitrile: 0, 0.23, 2.3, 23, 230, 2300 µg/mL
32 Negative controls: Vehicle +S9 mix, cell suspensions ±S9 mix
33 GLP: Yes
34 Study period: 1978 – 1979, report dated 1980

36 Vantocil IB, dissolved in DMSO, was investigated over a dose range of 0.25 – 2500 µg/mL in
37 experiment I and 25 – 3000 µg/mL in experiment II in quadruplicate cultures. All experiments
38 were conducted in the presence of S9 from livers of Aroclor 1254 induced Sprague-Dawley rats.
39 Negative controls consisted of the vehicle DMSO + S9 mix or of cell suspensions ±S9 mix.
40 Benzidine and Acrylonitrile were used as positive controls in experiment I and II, respectively.
41 Cell suspensions were incubated at room temperature for 3 hr after addition of a metabolic
42 activation system and test solutions were added and incubated at room temperature for 3 hr.
43 After transfer to petri dishes, survival or transformation of cells was examined after an
44 incubation period of either 6 – 9 or 14 – 21 days in a humidified atmosphere.

Results

47 Cytotoxicity was observed in the survival and transformation assay at concentrations of 250
48 µg/mL and above. The number of transformed cell colonies did not differ between plates
49 containing the test substance and those containing the negative controls. The positive controls

1 induced transformed colonies demonstrating the sensitivity and suitability of the test system.
2 The study authors concluded that Vantocil IB did not induce cell transformations in baby
3 hamster kidney fibroblasts (BHK21/C13) in the presence of S9-mix when tested up to cytotoxic
4 concentrations.

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8 Ref.: 92

9 **SCCS Comment**

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

11 **3.4 Exposure assessment**

12
13 The applicant proposed assessing aggregate exposure to PHMB using refined aggregate
14 exposure models based on real habits and practices data, probabilistic modelling techniques
15 that simulate amount per use data from full distributions and the presence and concentration of
16 PHMB for different product categories. Since a probabilistic approach for aggregate exposure
17 has not yet been agreed upon by the SCCS, a standard deterministic exposure assessment has
18 been carried out.

19 Ref.: 118

20 **3.5 Safety evaluation (including calculation of the MoS)**

21
22 On the basis of the newly generated dermal penetration data, the "residual stratum corneum
23 + epidermis" fractions were not considered as contributing to the SED. The application of this
24 approach to PHMB is supported by the 9th Revision of the Notes of Guidance (SCCS, 2016),
25 stating that in the case of substances with very low dermal absorption and limited permeation,
26 the epidermis may be excluded when it is demonstrated that no movement of the chemicals
27 from the skin reservoir to the receptor fluid occurs. With respect to potential contribution of
28 exposure from non-cosmetic use to the overall exposure, the SCCS also takes into
29 consideration the EU Biocidal Products Committee (BPC) Opinion on PHMB. On the basis of the
30 final Opinion issued by the BPC on 16/17 June 2015, it is considered that the approval of any
31 consumers' related biocidal application has a very low probability of being accepted. This is
32 further supported by the wording within the Biocidal Products Committee Opinion on the
33 various product types. On this basis, the actual future consumer related exposure to PHMB is
34 considered in practical terms to be very low. The BPC Opinion has major implications for
35 potential consumer exposures and will result in a significant reduction and potential elimination
36 of such exposures by July 2017. With respect to interim as well as residual exposures, the
37 additional exposure from non-cosmetic use for the present to beyond July 2017 is estimated to
38 be 0.00161 mg/kg bw in the worst-case scenario. Even if this amount is added to the systemic
39 exposure from cosmetic use, the risk assessment will still result in an acceptable Margin of
40 Safety (MoS) of 227. The use of PHMB in contact lens care solutions falls under the Medical
41 Device Regulations. PHMB may be present in these solutions at 1-2 ppm (0.0001-0.0002%).
42 PHMB has a small market share in this application and coupled with the very low levels of
43 PHMB present, the overall contribution to consumer exposure is considered to be insignificant
44 for this product.

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46 Ref.: 119, 5

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CALCULATION OF THE MARGIN OF SAFETY

(Dap= dermis+ receptor fluid+ 1SD)

Absorption through the skin DAp (%) =	4.09 %
Amount of cosmetic product applied daily A (g/d) =	17.4 g/d
Concentration of ingredient in finished product C (%)=	0.1%
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 =	0.012 mg/kg bw/d
No adverse observed effect level NOAEL =	36 mg/kg bw/d
(104 week oral rat study)	
Corrected NOAEL based on 8.5 % oral absorption =	3.1 mg/kg bw/d

MOS = Corrected NOAEL/SED

Margin of Safety	adjusted NOAEL/SED = <u>258</u>
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+ exposure from non-cosmetic use

Absorption through the skin DAp (%) =	4.09 %
Amount of cosmetic product applied daily A (g/d) =	17.4 g/d
Concentration of ingredient in finished product C (%)=	0.1%
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 =	0.012 mg/kg bw/d
+ exposure form non-cosmetic use : 0.00161 mg/kg/d =	0.01361 mg/kg bw/d
No adverse observed effect level NOAEL =	36 mg/kg bw/d
(104 week oral rat study)	
Corrected NOAEL based on 8.5 % oral absorption =	3.1 mg/kg bw/d

MOS = Corrected NOAEL/SED

Margin of Safety	adjusted NOAEL/SED = <u>227</u>
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3.6 Discussion:

Physico-chemical properties

PHMB is a polymer which in neat form represents a solid/powder of > 94.2 % purity. It has a 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. Up to 6% of the commercially used mixture is present as monomers, i.e. as constituent with a molecular weight below 500 g/mol.

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1 **Function and uses**

2
3 PHMB is supported under Directive 98/8/EC for uses as a disinfectant and it is used as a
4 preservative and as an antimicrobial agent. As a preservative, PHMB is used in cosmetics,
5 personal care products, fabric softeners, contact lens solutions, hand washes, and more.

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

11 PHMB is also used to preserve wet wipes; to control odour in textiles; to prevent microbial
12 contamination in wound irrigation and sterile dressings; to disinfect medical/dental utensil and
13 trays, farm equipment, animal drinking water, and hard surfaces for food handling institutions
14 and hospitals; and to deodourise vacuums and toilets. PHMB is used in antimicrobial hand
15 washes and rubs and air filter treatments as an alternative to ozone.

16 PHMB is also used as an active ingredient for recreational water treatment, as a chlorine-free
17 polymeric sanitiser, which is effective against a wide variety of microorganisms.

18 Further reported uses of PHMB are purification of swimming pool water, beer glass sanitisation,
19 solid surface disinfection in breweries and short-term preservation of hides and skins.
20
21

22 **Toxicological Evaluation**

23
24
25 **Acute toxicity**

26
27 From two acute oral toxicity studies performed with PHMB in rats, an LD₅₀ value of 1049 mg/kg
28 bw was reported in one of the studies and LD₅₀ values of 549 mg/kg bw for males and 501
29 mg/kg bw for females were reported for the other study. Based on these values, PHMB can be
30 considered of moderate acute oral toxicity; classification as Acute Tox 4 H302 (harmful if
31 swallowed) is justified.

32 Two acute dermal toxicity tests have been performed. No deaths occurred up to dose levels of
33 5000 mg/kg bw/d. Thus it can be concluded that PHMB is not acutely toxic by the dermal
34 pathway.

35 Two acute inhalation toxicity studies have been performed which were not available to the
36 SCCS for evaluation. No fatal cases in Wistar rats were observed when exposed by inhalation to
37 0.1% PHMB. Recently, RAC has identified one of the studies leading to LC50 values of 0.29
38 mg/l for males, 0.48 mg/l for females and 0.37 mg/l for males and females combined as key
39 study thus warranting classification as Acute Tox 2 H330 (fatal if inhaled) and indicating acute
40 inhalation toxicity. According to the rules on classification of mixtures (see ECHA Guidance on
41 the application of CLP criteria (SCCS Ref. III)) and under the assumption that all other
42 ingredients present in the finished cosmetics products can be considered as being not acutely
43 toxic; classification for a mixture containing 0.1 % PHMB would not be warranted.
44
45

46 **Irritation and corrosivity**

47
48 PHMB can be considered as irritating to skin. Whereas neat PHMB is corrosive to the eye, 20 %
49 solutions of PHMB can be considered as moderately irritating to the eye. PHMB can also be

Opinion on Polyaminopropyl Biguanide (PHMB) - Submission III

1 considered irritating to the respiratory tract leading to 50 % reduction of respiratory rate at
2 264 mg/m³.

3
4
5 ***Skin sensitisation***
6

7 Based on various guinea pig maximization- and Buehler tests, PHMB can be considered as a
8 moderate to strong sensitiser in animals. The threshold for eliciting skin reactions or elicitation
9 in guinea pigs is approximately 1 %. From HRIPT tests it can be concluded that a concentration
10 of 2 % PHMB is capable of causing skin sensitisation humans which can be elicited at
11 concentrations starting from 0.2 % a.i. From reports on patch tests PHMB sensitisation in
12 humans can be considered of low frequency (up to 0.5 % in dermatitis patients).

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

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17 ***Dermal Absorption***
18

19 The newly submitted studies on percutaneous penetration of PHMB were in compliance with
20 SCCS requirements and GLP principles. The substance was tested in two representative
21 cosmetic formulations: aqueous micellar solution and in oil in water emulsion. An additional
22 extended observation time of 72 h was performed. The study showed that there was no
23 movement of the PHMB from the skin reservoir to the receptor fluid. For that reason the
24 amount found in the epidermis should not be taken into the final calculation of the bioavailable
25 dose. This is in line with SCCS/1564/15.

26 SCCS notes that tape stripping was performed with 5 tape strips as advised. The lower layers
27 of stratum corneum were left and taken together into calculation with the rest of the epidermis.
28 The chosen cut-off value of 4 kΩ for barrier function assessment is not of common practice
29 (threshold of 10 kΩ is normally used) and may lead to an overestimation of the results.

30
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32 ***Toxicokinetics***
33

34 From a properly conducted oral (gavage) single dose toxicokinetic study using different
35 molecular weight fractions of PHMB, the low molecular weight fraction might be considered as
36 the most bioavailable fraction of which males excreted 7.8 % via urine and 94.1 % via feces
37 and females excreted 2.6 % via urine and 93.5 % via feces. In tissues, highest amounts of
38 radioactivity were found in the livers and kidneys. The residual carcasses contained 0.22 and
39 0.28 % of the dose. From this study, up to 8.5 % of the applied radioactivity might be
40 considered bioavailable (sum of urinary excretion and radioactivity in tissues and residual
41 carcass at study termination).

42 In a repeated dose dietary study, the principle route of excretion was also feces. Urinary
43 excretion amounted to 2.3 % and bioavailability amounted to 4.7 %. The major parts of
44 radioactivity were excreted during the first 24 hr and excretion was virtually complete within 72
45 hr. The SCCS does not consider PHMB to belong to substances with low bioavailability as
46 absorption percentages of total radioactivity up to 8.5 % were observed in oral studies.

47 The SCCS notes, that absorption rates up to 8.5 % of the applied dose are quite high for a
48 polymeric substance. However, as proper structural identification has neither been performed
49 in the oral TK nor in the dermal absorption studies (only total radioactivity has been

1 determined in these studies), high absorption rate for the polymer could be due to its
2 degradation products.
3
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5 **Repeated dose toxicity**

6
7 Repeated dose toxicity studies using different durations (3 weeks up to 2 years) and different
8 application routes (oral – drinking water; oral – diet; dermal; inhalation) have been performed
9 in different animal species (rat, mouse, Beagle dog).

10 Short-term and subchronic oral studies mainly served as range finder for studies of longer
11 duration. Subchronic dietary studies performed in rats and mice caused reductions in body
12 weight and effects on weight gain. In rats, the kidney was identified as the target organ; there
13 were changes in renal function as well as changes in some plasma parameters. However,
14 interpretation of the dietary subchronic studies was hampered by limited histopathologic
15 examination.

16 In a dietary 12-month study in Beagle dogs, a NOAEL of 54 mg/kg bw was derived. At the
17 highest dose tested there were adverse effects on scrotal skin, indications of liver impairment
18 and histopathological findings in skin, liver and – for males only – in kidneys and testes. In a
19 dietary 2-year rat study NOAELs of 36 and 45 mg/kg bw/d were derived for male and female
20 animals, respectively. At the highest dose tested, most prominent findings were reductions in
21 body weight, changes in plasma parameters, the incidence of hepatocyte fat and spongiosis
22 hepatis. Further, the incidence of haemangiosarcomas was increased at the highest dose
23 tested.
24
25

26 **Reproductive toxicity**

27
28 In an oral chronic study in Beagle dogs, histopathologic changes in testes were observed at the
29 highest dose tested (169 mg/kg bw/d, reduced to 108 mg/kg bw/d). In a 2- and a 3-
30 generation reproductive toxicity study in rats, no adverse effects on the reproductive
31 parameters addressed were observed. No evidence of foetal/developmental toxicity
32 independent from maternal toxicity was observed in developmental toxicity studies in rats and
33 rabbits. There were indications for retarded ossification at 20 and 40 mg/kg bw/d in a
34 developmental toxicity study in mice, which was not considered an adverse effect by the SCCS.
35
36

37 **Mutagenicity / genotoxicity**

38
39 The bacterial reverse mutation test is considered not suitable to be used as PHMB is a
40 bactericide. PHMB was negative in a well performed gene mutation assay in mammalian cells
41 and in an *in vitro* chromosome aberration test in human lymphocytes. An *in vitro* micronucleus
42 test was also negative but only short treatment times were used. The negative results found in
43 *in vitro* tests were confirmed under *in vivo* conditions as PHMB did not lead to the induction of
44 micronucleated polychromatic erythrocytes in mice. These negative results in the *in vivo*
45 micronucleus test were further supported by a negative *in vivo* UDS test.

46 Consequently, based on the available data PHMB can be considered to have no genotoxic
47 potential and additional tests are unnecessary.
48

49 When the purity of PHMB used is considered, 6% of monomer is present.

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1 The exposure to the monomer in a 20% dilution of PHMB with an absorption value of 4.09 % is
2 0.00668 µg/kg bw/d= 6.68 ng/kg bw/d.
3 Both monomers from which PHMB has been synthesised are present in the mixture < 500
4 daltons. Worst case assumption would be that only these monomers are present. Both are
5 non-genotoxic. For a substance of Cramer Class III without genotoxicity alerts, a TTC value of
6 90 µg/person/d, corresponding to 1.5 µg/kg bw/d, is recommended in the SCCS Notes of
7 Guidance.

8
9
10 **Carcinogenicity**

11
12 In a rat oral chronic toxicity/carcinogenicity assay, an increased incidence of
13 haemangiosarcomas (statistically not significant) was observed at the highest dose of 2000
14 ppm PHMB. Recently it has been concluded by ECHA's RAC committee that evidence from the
15 chronic rat study is not sufficient to demonstrate a clear treatment-related effect. In an oral
16 study in mice, PHMB increased the incidence of vascular tumors, mainly in the liver. In a
17 dermal study in mice, a statistically significant increase in the incidence of haemangiosarcomas
18 was observed in females at the highest dose (750 mg/kg bw/d; considered to exceed MTD)
19 tested. Despite the weak evidence of the carcinogenic potential of PHMB, RAC recently
20 concluded that classification as Carc 2 H351 (suspected of causing cancer) according to CLP
21 would be appropriate.

22
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24 **Photo-induced toxicity**

25
26 No studies on photomutagenicity or photoclastogenicity have been performed. PHMB cannot be
27 considered as a photosensitiser.

28
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30 **Human data**

31
32 See sensitisation.

33
34
35 **Special investigation**

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39 **Safety evaluation**

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41 SCCS used repeated dose toxicity for NOAEL setting and did not make a calculation based on
42 carcinogenic effects as PHMB is not a genotoxic carcinogen.

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4. CONCLUSION

1. *In light of the new data provided, does the SCCS consider Polyaminopropyl Biguanide (PHMB) safe when used as preservative in all cosmetic products up to a maximum concentration of 0.1%?*

In the previous Opinion, the SCCS stated that the Polyaminopropyl Biguanide (PHMB) is not safe up to maximal concentration of 0.3% and the safe use could be based on a lower use concentration and/or restrictions with regard to cosmetic products' categories. In order to ensure the safe use of PHMB at a concentration lower than 0.3%, the applicant presented new dermal absorption studies on additional representative cosmetic formulations.

Based on the data provided, the SCCS is of the opinion that the use of Polyaminopropyl Biguanide (PHMB) as a preservative in all cosmetic products up to 0.1% is safe.

2. *Alternatively, taking into account the EU market data available, does the SCCS consider Polyaminopropyl Biguanide (PHMB) safe when used as preservative up to a maximum concentration of 0.1% in all cosmetic products with the exclusion of those products categories (body lotion, hand cream and oral care) in which this ingredient is seldom used?*

Not applicable.

3. *According to the data available, does the SCCS consider Polyaminopropyl Biguanide (PHMB) safe for use in sprayable formulations up to a maximum concentration of 0.1%?*

As no new safety data on inhalation is available on PHMB, its use in sprayable formulations is not advised.

4. *Does the SCCS have any further scientific concerns with regard to the use of Polyaminopropyl Biguanide (PHMB) in cosmetic products?*

The SCCS does not have any further concerns.

5. MINORITY OPINION

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