Committee for Risk Assessment
RAC

Annex 1

Background Document

to the Opinion proposing harmonised classification and labelling at Community level of

Polyhexamethylene biguanide or Poly(hexamethylene) biguanide hydrochloride or
PHMB

ECHA/RAC/CLH-O-0000001973-68-01/A1

Polyhexamethylene biguanide or Poly(hexamethylene) biguanide hydrochloride or
PHMB
EC Number: not allocated (polymer)
CAS Number: 27083-27-8 or 32289-58-0

9 September 2011

CONTENTS

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: polyhexamethylene biguanide or poly(hexamethylene) biguanide hydrochloride or PHMB

EC Number: not allocated (the substance was not notified under Directive 92/32/EEC)

CAS number: 27083-27-8 or 322289-58-0

Registration number (s): -

Purity: > 94.2% w/w in dry weight

Impurities: This information is confidential and then provided in confidential part of the dossier provided in appendix 1.

PHMB is a polymer. It is normally supplied as an aqueous solution of 20% PHMB (VANTOCIL TG). However, PHMB exists also as another technical grade, called solid PHMB (98% w/w PHMB content for the typical concentration). According to the definition of a "substance" under REACh, the proposed entry is referring to the "pure" PHMB with a purity expressed in dry weight.

The vast majority of the studies were generated using VANTOCIL TG (or equivalent materials), but some toxicological studies were also carried out with solid PHMB. The specifications of the substance are presented for both solid PHMB and VANTOCIL TG in appendix 1.

Both sources of PHMB are considered relevant to evaluate hazardous properties of the proposed entry on "pure" PHMB. In particular, PHMB supplied in aqueous solution of 20% has a slightly lower average molecular weight than solid PHMB and toxicokinetic data show that oral absorption increase with decreasing molecular weight fractions. All studies by oral route were performed with the aqueous solution of PHMB, which is considered to maximise internal exposure to PHMB. For local toxicity, most tests were also available with solid PHMB.

Proposed classification based on Directive 67/548/EEC criteria:

Xn; R22
T+; R26
Xi; R41
Xi ; R43
T; R48/23
Carc. Cat. 3 ; R40
N, R50/53

Proposed classification based on CLP criteria:

Hazard statements:

Acute Tox 4 – H302
Acute Tox 1 – H330
Eye Damage 1 – H318
Skin Sens 1B – H317 according to 2nd ATP to CLP Regulation
STOT RE 1 – H372 (respiratory tract) (inhalation)
Carc 2 – H351 (default)
Aquatic Acute 1 - H400
Aquatic Chronic 1 - H410

.

Proposed labelling:

According to CLP criteria:

Pictograms: GHS05, GHS 06, GHS08, GHS09.
Signal word: Danger
Hazard statements: H302, H330, H318, H317, H372,H351, H410

According to Directive 67/548/EEC criteria
Symbol(s): T+; N
R-phrases: R22 ; R26 ; R41 ; R43 ; R48/23 ; R40 ; R50/53
S-phrases: S22, S26, S36/37/39, S45, S60, S61

Proposed specific concentration limits (if any):

Under CLP, an M-factor = 10 is proposed.

[Under CLP (2nd ATP), an M-factor = 10 is proposed for both acute and chronic aquatic toxicity.]

Under Directive 67/548/EEC, SCL are proposed for environment:

Specific concentration limits :

$C \geq 2.5\%$	N, R50/53
$0.25\% \leq C < 2.5\%$	N, R51/53
$0.025\% \leq C < 0.25\%$	R52/53

Proposed notes (if any):

None

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

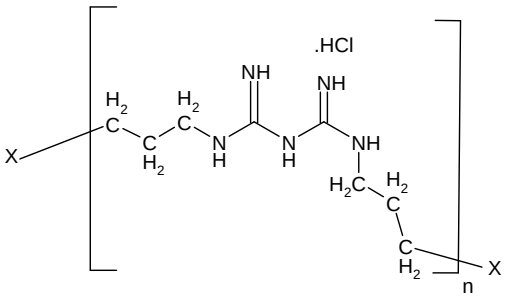
1.1 Name and other identifiers of the substance

Chemical Name:	Polyhexamethylene biguanide hydrochloride
EC Name:	Not allocated as the substance is a polymer (the substance was not notified under Directive 92/32/EEC)
CAS Number:	27083-27-8 and 32289-58-0 Two equivalent CAS number can be allocated depending on how the polymer is described. CAS-No 27083-27-8 expresses the PHMB in terms of its starting monomers (N,N’’’-1,6-hexanediylbis(N’-cyanoguanidine) and 1,6-hexanediamine). CAS-No 32289-58-0 expresses the PHMB as the resultant polymer.
IUPAC Name:	Polyhexamethylene biguanide hydrochloride

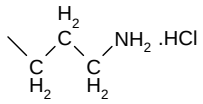
1.2 Composition of the substance

Chemical Name:	Polyhexamethylene biguanide hydrochloride
EC Number:	Not allocated as the substance is a polymer (the substance was not notified under Directive 92/32/EEC)
CAS Number:	27083-27-8 and 32289-58-0.
IUPAC Name:	Polyhexamethylene biguanide hydrochloride
Molecular Formula:	$(C_8H_{17}N_5)_n.nHCl$, n=1-40

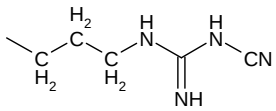
Structural Formula:



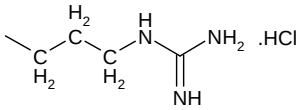
Where X =



Or X =



Or X =



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

Information on impurities is confidential and then provided in a confidential part of the dossier in appendix 1.

Physico-chemical properties

REACH ref Annex, §	Property	Value	[enter comment/reference or delete column]
VII, 7.1	Physical state at 20°C and 101.3 KPa	Off white to pale yellow powder with strong ammonia smell.	Sudworth, 2002
VII, 7.2	Melting	78.9-136.3ºC	Bannon, 2008
VII, 7.3	Boiling point	The substance decomposes at 205-210°C before boiling	Field, 1991
VII, 7.4	Relative density	1.20 ± 0.0025 (20°C ± 0.5°C)	Sudworth, 2002
VII, 7.5	Vapour pressure	1.32 x 10^{-7} Pa (20°C) 4.11 x 10^{-7} Pa (25°C)	Chang, 2008
VII, 7.6	Surface tension	68.5 ± 0.6 mN/m temperature: 25 ± 0.5°C	Schofield, 2007
VII, 7.7	Water solubility	41% w/w ± 1% ≈ 700 g/L temperature: 25 ± 1°C	Sudworth, 2002
VII, 7.8	Partition coefficient n-octanol/water (log value)	Log P_{ow} = -2.3 (experimentally estimated) temperature: 25ºC ; pH: 7.4	Bowhill, 2007
VII, 7.10	Flammability	Not flammable	Schofield, 2007
VII, 7.11	Explosive properties	Not explosive	Schofield, 2007.
VII, 7.12	Self-ignition temperature	No Ignition Below 400°C (Upper Limit of Test)	Schofield, 2007.
VII, 7.13	Oxidising properties	Not oxidising	Schofield, 2007
XI, 7.16	Dissociation constant	pKa=4.19 at 25°C	Field, 1991
	Reactivity towards container material	Experience in use indicates no reactivity with container materials.	Field, 1991
	Thermal stability	Onset of Decomposition: 205 to 210 °C	Field, 1991
	Solubility in organic solvents	Methanol : 41% w/w ±1% at 25 ± 1°C Ethanol : 0.5% w/w ± 0.08% at 25 ± 1°C Acetone: 2.7 ppm at 22°C Dichloromethane: 0.2 ppm at 22°C Ethyl Acetate : = 0.1 ppm at 22°C Toluene: 0.2 ppm at 22°C n-hexane: 0.1 ppm at 22°C Acetonitrile: 0.8 ppm at 22°C	Field, 1991

Table 1: Summary of physico- chemical properties

2 MANUFACTURE AND USES

Not relevant for this dossier.

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

PHMB is not classified according to Annex I of Directive 67/548/EEC or to Annex VI of CLP Regulation.

3.2 Self classification(s)

Two different classifications were proposed by two different notifiers in the scope of the Biocidal Product Directive (98/8/CE). However, only one was dealing with solid PHMB:

Xn; R22
Xi; R37/38
Xi; R41
Xi; R43
N ; R50/53

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation

4.1.1 Stability

4.1.1.1 Hydrolysis

Hydrolysis study following the OECD guideline 111 (reliability = 1) was performed with pH 4, 7 and 9 at 50°C as a preliminary study (**Sudworth, 2006**). Less than 10% hydrolysis was found after 5 days for all pHs tested**.** Consequently, according to the OECD guideline 111, PHMB is considered to be hydrolytically stable.

4.1.1.2 Photolysis

According to OECD guideline 316, direct photolysis can be an important dissipation pathway for some chemical pollutants that exhibit significant light absorption above the 290 nm cutoff of solar irradiation at the earth's surface and as PHMB absorption spectra maximum was not found in visible wavelength, PHMB could be considered like non photodegradable. No valid study concerning this endpoint is available.

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

4.1.2.2 Screening tests

A ready biodegradation test was performed according to OECD guideline 301B (**Long and Roberts, 1994**; reliability = 2)**.** The biodegradation of two concentrations of ^{14}C-PHMB (0.1 and 1.0 mg .L^{-1}) was investigated and $^{14}CO_2$ emission as mineralization was measured for 99 days. The inoculum came from a secondary effluent. Comparing to the NOEC value reported for microorganisms (section 7.4.1, NOEC=12 mg PHMB l^{-1}), these tested concentrations should therefore not be toxic for microorganisms. After 99 days, only 3.8% of PHMB is mineralised to CO_2 thus this substance is considered as **non readily biodegradable** in the strict terms of OECD 301B guideline.

Mineralisation in seawater was also investigated (**Mac Lean *et al.*, 2005a**; reliability = 2). After 56 days, at concentrations of 1 and 0.1 mg PHMB l^{-1}, 2.6% and 10.1% CO_2 mineralisation was observed respectively. For the highest concentration, some evidence of toxicity was noticed and could explain the lower level of mineralisation.

4.1.2.3 Simulation tests

A simulation test according to OECD 303A guideline (reliability = 2) was conducted to investigate PHMB degradation in conditions imitating a domestic sewage treatment plant (**MacLean *et al.*, 2005b**). For up to 144 days, ^{14}C-PHMB degradation was tested continuously into a system which was allowed to reach a steady state of activity, and total radioactivity in the effluent,

waste solids and CO_2 traps monitored and analysed. The vessels were inoculated with domestic activated sewage sludge (total filterable solids 903 mg l^{-1}) and dosed initially with a nominal ^{14}C-PHMB concentration of 50 µg l^{-1} for 53 days. Following this period, the unit was dosed at increasing nominal concentrations of 150, 250 and 500 µg l^{-1} for a further 14 days, 14 days and 63 days, respectively. Overall, during the 144 day period, <1% of the applied radioactivity was evolved as $^{14}CO_2$. 18% of the applied radioactivity was measured in the aqueous effluent, and the residual 82% was sorbed onto the sludge biomass. Therefore, under conditions which more closely simulate the actual conditions in a Sewage Treatment Plant, PHMB is very slightly mineralized to CO_2. The water discharge observed is caused only by a modification of PHMB distribution related to its property of adsorption leading to an accumulation of this active substance in activated sludges (**Gilbert et al., 2005**).). The discrepancy between the log Pow value (-2.3) and the ability of PHMB for strong sorption could be explained by the nature of this substance. Indeed, PHMB is a cation and the value of the experimental partition coefficient Log Pow is out of the domain for the logKoc calculation (**Sabljic & Güsten, 1995**)

4.1.3 Summary and discussion of persistence

According to the criteria for degradation in the guidance to Regulation EC n° 1272/2008 on CLP, PHMB was found to be not readily biodegradable and slight rates of mineralization were found in water (see Table 2). This substance is a mixture of polymers and biodegradation of polymers cannot be evaluated only regarding mineralization. Even if mineralization rates are low, a transformation of PHMB might occur in water and soil and the whole process of transformation, primary degradation as well as mineralization, should be taken into account. The biotic degradation of polymers includes several steps (biodeterioration, depolymerisation, assimilation and mineralisation) and the process can stop at each stage producing metabolites which might be more or less toxic than PHMB or modifying the chemical distribution of these polymers. Therefore, studying primary biodegradation is found to be complicated due to a limited feasibility for extraction, separation and analysis of PHMB polymers. Nevertheless, **O'Malley et al. (2006)** assessed the biodegradability of end-groups of PHMB using a reductive approach with model compounds representing the three possible end-goups of PHMB. Bacteria were screened for growth at the expense of each model compound (at non-inhibitory concentrations) as sole nitrogen source. Several bacteria were shown to utilise amine or guanidine end-groups, although the cyanoguanidine end-group remained unexploited and seemed to be recalcitrant. However, if PHMB degradation should proceed via a progressive degradation from the ends of the molecules, the presence of cyanoguanidine end-groups may inhibit biodegradation for at least a fraction of the PHMB molecules within heterogeneous mixture. Furthermore, **O'Malley et al. (2007)** showed that even with selected and adapted strains, in laboratory conditions and enriched medium, the biodegradation of PHMB reached only 29 % after 35 days confirming that PHMB is not easily and weakly biodegradable.

Following the CLP criteria , PHMB is considered not rapidly degradable as the level of degradation based on carbondioxide generation did not reach the 60% of the theoretical maximum within the 10 days window.

4.2 Environmental distribution

4.2.1 Adsorption/desorption

Not relevant for this type of dossier

Table 4.2: Biodegradation of PHMB

Reference	Guideline / Test method	Test type	Test parameter	Inoculum			Additional substrate	Test substance concentr.	Degradation	
				Type	Concen-tration	Adap-tation			Incubation period	Rate [%]
PHMB: Aerobic biodegradation in water *(Long and Roberts, 1994)*	OECD 301B	Aerobic ready	$^{14}CO_2$ evolution	Secondary effluent	N/A	No	No	0.1 or 1.0 mg C l^{-1} as PHMB	99 days	3.8 % mineralisation
PHMB: Biodegradability in seawater *(Mac Lean* et al., *2005)*	OECD 306	seawater	$^{14}CO_2$ evolution	Seawater	N/A	No	No	0.1 or 1.0 mg l^{-1} equivalent to 0.05 or 0.5 mg C l^{-1}	56 days	10.1% mineralisation
PHMB: Aerobic sewage treatment simulation and chronic toxicity of treated effluent to *Daphnia magna* *(MacLean* et al., *2005)*	OECD 303A	Sewage treatment simulation	$^{14}CO_2$ evolution ^{14}C-PHMB removal	Activated sludge and fresh settled sewage	Suspended solids 903 mg l^{-1}	No	No	0.5 mg l^{-1}	144 days	<1% mineralisation Distribution: 18% discharged in aqueous effluent 82% sorbed to sludge biomass

4.2.2 Volatilisation

Not relevant for this type of dossier

4.2.3 Distribution modelling

No relevant data available.

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

Based on its log Kow = -2.3 no concern over any potential for bioaccumulation could be concluded. Furthermore, the low Kow, the high molecular weight (PHMB>700 g/mol) may indicate the substance unlikely to bioaccumulate.

4.3.1.2 Measured bioaccumulation data

No relevant data available

4.3.2 Terrestrial bioaccumulation

Not relevant for this type of dossier

4.3.3 Summary and discussion of bioaccumulation

See 4.3.1.1

4.4 Secondary poisoning

Not relevant for this type of dossier

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

PHMB is poorly absorbed by oral route with an absorption rate around 4%. Three days after administration of a single dose in water or in food 0.2 to 7.8% of PHMB has been excreted in urine and 0.2 to 1.3% is detected in the carcass with the liver and kidneys having the highest concentrations. The highest oral absorption and urinary excretion are observed when animals are given a low molecular fraction of PHMB. A study with cannulated rats found that only 0.2% of administered PHMB was excreted in bile (**Lythgoe, 1995a and 1995b,** purity: 20% in aqueous solution).

In vitro, a low dermal absorption of PHMB has been measured on human epidermis either at ambient temperature (0.021 to 0.146% of the dose was absorbed after 96h depending on the solution concentration) (**Clowes, 1996**, purity: 20.2% in aqueous solution) or at 40°C (0.007% after 24h) (**Clowes, 1998**, purity: 20.2% in aqueous solution).

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

In a first study, eight groups of 5 male and 5 female rats were fasted for 16-20 hours and then given various doses of a 20% aqueous solution of PHMB **(Jackson, 1979)**. The test article was administered by stomach tube and the animals were observed for 14 days. A standard volume of 10 ml/kg was dosed to each animal. The study is considered equivalent to an OECD 420 study with the deficiency that animals were not subjected to gross necropsy.
Clinical signs of toxicity, present in all groups, were salivation, lacrymation, piloerection and in isolated cases, a subdued appearance. These signs did not persist beyond day 7 or 8 of the study.
The oral LD_{50} of the 20% aqueous solution is 2.7 g/kg in males and 2.5 g/kg in females, equivalent to 549 mg PHMB/kg in males or 501 mg PHMB/kg in females.

In another acute oral study, a total of 6 female animals were dosed individually in sequence (**Driscoll, 2003a**). All animals were dosed once only by gavage, at dose levels of 2000 or 550 mg PHMB /kg. The test material was administered orally as a solution of the solid material dissolved in distilled water. All surviving animals were observed for 14 days post-dose. The study was performed according to guideline OECD 425 and GLP.
All three animals treated with 2000 mg/kg were found dead during the day of dosing or one day after dosing. No deaths were noted at a dose level of 550 mg/kg.
Hunched posture and pilo-erection were noted in 2 animals treated with 2000 mg/kg just after dosing. Signs of systemic toxicity also noted 1 day after dosing in 1 animal treated with 2000 mg/kg were lethargy, ataxia, decreased respiratory rate, laboured respiration, ptosis and tiptoe gait. There were no signs of systemic toxicity noted in animals treated with 550 mg/kg. The surviving animals showed expected gains in bodyweight over the study period. Abnormalities noted at necropsy of animals that died during the study were haemorrhagic or abnormally red lung, dark liver, dark kidneys, haemorrhage or sloughing of the gastric mucosa, sloughing of the non-glandular epithelium of the stomach and haemorrhage of the small intestine. No abnormalities were noted at necropsy in animals that survived through the 14-day observation period.
The acute oral LD_{50} is 1049 mg PHMB/kg.

5.2.2 Acute toxicity: inhalation

No acute inhalation study is available on PHMB.

However, a 28-day inhalation study (**Carney, 1976**) provides information that is relevant to evaluate acute toxicity of PHMB by respiratory route. The study was performed before adoption of guidelines and its interpretation was limited by poor reporting (see 5.5.2).

SPF albino rats (4/sex/group) were exposed to atmospheres containing respirable particles of PHMB (prepared from aqueous solution of PHMB 20%; concentrations expressed as concentrations of respirable particles with mass mean diameter < 7 µm) at concentrations of 26, 12.5, 2.75, 0.25 and 0.025 µg/L PHMB for 6 hours per day for 5 days a week, for three weeks, snout-only.

In the high dose group, very severe nasal irritation and marked dyspnoea were noted ante-mortem, only a single exposure was possible and all treated rats died within 24 hours of first exposure. The concentration of 12.5 µg/L respirable particles proved particularly toxic. Severe nasal irritation and dyspnoea were evident and all rats died following the fourth exposure period. Mortality was also observed at 2.75µg/L, where 3 males and 1 female died during or after the sixth exposure.

The LC_{50} can therefore be estimated, on the basis of limited available data, as smaller than 26µg/L for a single 6 hour exposure to rats.

NOTE: In August 2011, CEFIC requested RAC to consider a set of studies from 1999 not submitted during the public consultation. One study on acute inhalation (Kilgour, 1999) was considered potentially relevant. A summary of the acute inhalation study is presented here: In an acute inhalation study **(Kilgour, 1999)** 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 LC_{50} 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.

5.2.3 Acute toxicity: dermal

A summary of the main results on acute toxicity of PHMB by dermal route is provided in Table 3. No classification is proposed for this route since no data are available to demonstrate that acute toxicity range estimate is ≤2000 mg/kg.

Table 3: Summary of PHMB acute dermal toxicity studies

Route	**Method** **Guideline**	**Species** **Strain** **Sex** **no/group**	**Dose Levels** **Duration of Exposure**	**Value** **LD50 or LC50**	**Reference**
Dermal	OECD 402 Test substance:	Rat;	5000 mg/kg (purity 96%)	> 5000 mg/kg (no mortality, signs of	Driscoll, 2003c

Route	Method Guideline	Species Strain Sex no/group	Dose Levels Duration of Exposure	Value LD50 or LC50	Reference
	solid PHMB (96%)	5/sex/group		irritation)	
Dermal	OECD 402 Test substance: 20% aqueous solution PHMB	Rat; 5/sex/group	400 mg/kg	> 400 mg/kg (no mortality or clinical signs)	Richeux, 2002a

5.2.4 Summary and discussion of acute toxicity

No acute inhalation study is available on PHMB. However, results from a 28-day study on PHMB as liquid aerosol (Carney, 1976), in which mortality was observed after a single exposure, showed that LC_{50} is estimated to be less than 26µg/L for a 6 hour exposure to rats. Extrapolation of this result to a 4-hour period can be made as recommended in IR/CSA section R7.4.4.1 (ECHA, 2008) using a modification of Haber's law ($C^n.t=k$). As *n* value is not available in the literature for PHMB and extrapolation is made to a shorter duration a default value of n=3 is used. The resulting estimated LC_{50} for a 4-hour exposure is 0.030 mg/l and warrants a classification **T+; R26** (CLP Acute Tox 1 – H330).

In August 2011, CEFIC requested RAC to consider an acute inhalation study (Kilgour, 1999). It is not possible to establish an LC_{50} 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.

RAC cannot explain with certainty the dissimilar results of both tests. Possible reasons could be the use of different rat strains, different vehicles and the generally few animals used in these studies.

For this reason and in line with the CLP guidance, RAC is of the opinion that the lowest value should be the basis for classification and therefore concludes that a classification **T+; R26** (CLP Acute Tox 1 – H330) is warranted based on the results from the study by Carney (1976).

For comparison, criteria for this level of classification are:
LC_{50} for aerosols or particulates ≤ 0.25 mg/l/4h according to the DSD (T+; R26)
and for inhalation (mist) LC_{50} ≤ 0.05 mg/l according to CLP (Acute Tox 1 – H330).

As presented above, acute toxicity of PHMB has been assessed by oral route in one study on a 20% solution of PHMB and in one study on pure PHMB in solid form (dissolved in water for administration), which are summarised in Table 4.

Table 4: Summary of PHMB acute oral toxicity studies

Route	Method Guideline	Species Strain Sex no/group	Dose Levels Duration of Exposure	Value LD50 or LC50	Reference
Oral	Equivalent to OECD 420.	Rat; 5/sex/group	700, 1000, 1500, 2000, 2500, 3000, 3500, and 5000 mg/kg of a 20% aqueous solution of	Males 549 mg PHMB/kg; Females	Jackson, 1979

Route	Method Guideline	Species Strain Sex no/group	Dose Levels Duration of Exposure	Value LD50 or LC50	Reference
	Test substance: 20% aqueous solution PHMB		PHMB	501 mg PHMB/kg	
Oral	US EPA 870.1100 OECD 425 Test substance: solid PHMB (96%)	Rat; Females; 3/group	550 or 2000 mg/kg;	1049 mg/kg	Driscoll, 2003a

Both studies by oral route indicate a moderate acute toxicity with LD_{50} between 500 and 1000 mg/kg in rats. RAC concludes that PHMB meets the criteria for classification in Acute Tox 4 – H302 (CLP Regulation (300 mg/kg ≤ LD_{50} ≤ 2000 mg/kg) and as Xn; R 22 (200 mg/kg ≤ LD_{50} ≤ 2000 mg/kg).

By dermal route, no mortality is induced by PHMB in rats at doses up to 5000 mg/kg and no classification is warranted.

5.3 Irritation

5.3.1 Skin

A summary of the main results on skin irritation of PHMB is provided in Table 5. The three studies with guideline conformity to OECD TG 404 were given priority and their mean values for erythema oedema were below 2.3 . Thus, no classification is proposed for skin irritation based on data available for pure PHMB.

Table 5: Summary of PHMB skin irritation animal studies

Species	Method Guideline	Average score 24, 48, 72 h		Reversibility	Result	Reference
		Erythema	Oedema			
Rabbit	Study conducted prior to guidelines (duration of exposure was 24 hours). Test substance: 20% aqueous solution PHMB	Average score 24-72 hours was 1.9 for intact skin and 1.8 for abraded skin.	Slight to moderate edema which subsided by 72 hours. Average score was 0.5 for intact skin and 0.8 for abraded skin	Yes.	Well defined to moderate erythema was observed in each animal at each skin site at 24 hours. This had subsided slightly at 72 hours. Average score was 2.3 for intact skin and 2. for abraded skin. Slight to moderate oedema was observed in all animals except one at 24 hours, but all signs of oedema subsided by 72	**Jackson, 1980a**

Species	Method Guideline	Average score 24, 48, 72 h		Reversibility	Result	Reference
		Erythema	Oedema			
					hours. By day 21 of the study there signs of scabbing and healing at the site of the abrasions.	
Rabbit	OECD 404 Test substance: solid PHMB (96%)	1.0	0.2	Yes.	There was no evidence of skin irritation following exposures of 3 minutes or 1 hour. After the 4 hour exposure the primary irritation index was 1.0. The mean 24-48-72h value for either erythema and eschar formation or oedema formation calculated for each animal tested is 1 or less. Following the 4-hour exposure well-defined erythema was noted at one treated skin site with very slight erythema at 2 treated skin sites 1 and 24 hours after patch removal. Very slight erythema was noted at all treated skin sites at the 48-hour observation and persisted at 1 treated skin site at the 72-hour observation. Slight oedema was noted at 1 treated skin site 1 hour after patch removal with very slight oedema at the 24 and 48-hour observation. There was no skin reaction at 7 days.	**Driscoll, 2003d**
Rabbit	OCDE 404 Test substance: 20% aqueous solution PHMB	2.0	0.0	Yes	24 hours after application moderate erythema was observed on the treated area of all three animals. The reaction was completely reversible between day 6 and 8. No oedema was observed.	Richeux, 2008
Rabbit	OCDE 404 Test substance: solid PHMB (96%)	0	0	-	No erythema or oedema observed at 1, 24, 48 or 72 hours.	Richeux, 2008a,

5.3.2 Eye

PHMB, as the solid material, was instilled into the eye of 1 rabbit at a volume of 0.1 ml (**Driscoll, 2003b**). The material was placed into the conjunctival sac of the right eye, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about 1 second immediately after treatment. Assessment of irritation was noted at 1 hour, 24 hours, 48 hours, 72 hours, 7 days, 14 days, and 21 days. The study was performed according to guideline OECD 405 and GLP.

Dulling of the normal lustre of the cornea was noted in the treated eye 1 hour after treatment. Scattered or diffuse corneal opacity was noted in the treated eye at the 24, 48, and 72-hour observations. Opalescent opacity was noted over approximately ½ the cornea of the treated eye with translucent corneal opacity in the remaining area at the 7-day observation. Opalescent opacity was noted over approximately ½ of the cornea of the treated eye with scattered or diffuse corneal opacity in the remaining area at the 14- and 21-day observation. Vascularisation, generalised in growth of vessels for approximately 2 mm, was noted in the treated eye at the 7, 14- and 21-day observations.

Iridal inflammation was noted in the treated eye 1 hour after treatment and at the 24, 48, and 72-hour time periods and at the 7 and 14-day time periods. Minimal conjunctival irritation was noted at the 21-day time period.

Severity scores are reported in Table 6 below.

Table 6: Individual Scores for Ocular Irritation

Rabbit Number and Sex	95 Male						
	Initial Pain Reaction = 2						
Time after Treatment	1 h	24 h	48 h	72 h	7 d	14 d	21 d
Cornea: - opacity degree - area covered	0d 3	1 3	1 3	1 3	3*V 2	3^{o}V 2	2V+ 2
Iris	1	1	1	1	1	1	0
Conjunctivae: - redness - chemosis - discharge	2 2 3	2P 3 3	2P 3 3	2P 3 3	2P 2 2	2P 2 2	1 1 1

d – Dulling of the normal lustre of the cornea
* - Grade 3 opacity over approximately ½ of the cornea with grade 2 opacity in the remaining area
V – Vascularisation, generalised ingrowth of vessels for approximately 2 mm
^{o} – Grade 3 opacity over approximately ½ of the cornea with grade 1 opacity in the remaining area
V+ - Vascularisation, ingrowth of vessels for approximately 5 to 6 mm
P – Pale appearance of the nictating membrane

Translucent corneal opacity, minimal conjunctival irritation and vascularisation were noted in the treated eye at the 21-day observation and were considered to be irreversible.

In another study (**Richeux, 2008b**), PHMB supplied as powder (purity 99.6%) was instilled into the eye of one New-Zealand rabbit at the dose of 0.1g.

At the conjunctival level, a moderate redness was noted 1 hour after instillation and still noted at the end of the observation at day 7. It was associated with an important chemosis noted 24 hours after instillation and still noted at the end of the observation. At the corneal level, a moderate opacity was registered 1 hour after instillation and still present at the end of the observation. At the iris level, congestion was registered from the 2nd day of the test and persisted until the end of the observation. An ulceration of the nictating membrane and the cornea was noted from the 1st day of the test. This lesion persisted for at least 72 hours.

Taking into account the severity of the reactions, the study was stopped at day 7 in accordance with the principles of animal welfare and additional animals were not treated.

Severity scores are reported in Table 7 below.

Table 7: Scores for Ocular Irritation

Rabbit Number and Sex	A9033			
Time after Treatment	24 h	48 h	72 h	Mean
Cornea opacity	2	2	2	2
Iris	1	1	1	1
Conjunctivae: - redness - chemosis	 3 3	 2 3	 2 3	 2.3 3

5.3.3 Respiratory tract

No specific study available. See acute toxicity by inhalation in 5.2.2.

5.3.4 Summary and discussion of irritation

Main results presented above for eye irritation are reported in Table 8 below.

Table 8: Summary of PHMB eye irritation animal studies

Species	**Method**	**Average score 24, 48, 72 h**				**Reversi-bility**	**Result**	**Reference**
		Cornea	Iris	Conjunctiva				
				Redness	Chemosis			
Rabbit	OECD 405; US EPA OPPTS 870.2400 Test substance: solid PHMB	24 h – 1 48 h – 1 72 h - 1	24 h – 1 48 h – 1 72 h - 1	24 h – 2 48 h – 2 72 h - 2	24 h – 3 48 h – 3 72 h - 3	No.	Solid PHMB produced irreversible ocular damage and was considered to be corrosive to the rabbit eye.	Driscoll, 2003b
Rabbit	OCDE 405 Test substance: solid PHMB	2	1	2.3	3.0	No (at day 7)	An ulceration of the nictating membrane and the cornea was noted from the 1st day of the test. This lesion persisted for at least 72 hours. Taking into account the severity of the reactions, the study was stopped at day 7.	Richeux, 2008b

In an eye irritation study performed according to guideline OECD 405, translucent corneal opacity, minimal conjunctival irritation and vascularisation were noted in the treated eye of a rabbit at the 21-day observation; effects were irreversible.

In a second test, effects were observed on cornea, iris and conjunctiva. Persistence and severity of the effect lead to cessation of the study at day 7. Ulceration of the nictating membrane and the cornea was also noted from the 1st day of the test and persisted for at least 72 hours.

The solid technical PHMB is therefore considered to be severely irritant to the rabbit eye due to irreversibility of effects and classification **Xi; R41** is warranted (CLP Eye damage 1 – H318).

No classification is required for skin or respiratory irritation.

5.4 Sensitisation

5.4.1 Skin

5.4.1.1 Animal data

Several animal studies are available and are described below. For each study, an evaluation of PHMB potency under the test condition was performed in accordance with section 3.4.2 of the CLP guidance.

The sensitisation potential of a 20.2% aqueous solution of PHMB was assessed in guinea pigs (**Duerden, 1993**). The study was performed according to OECD Guideline 406 and based on the method described by Magnusson and Kligman (Allergic Contact Dermatitis in the Guinea Pig. Pub Thomas, USA. 1970). Intradermal induction was performed with 0.06% PHMB in deionised water and Freund's complete adjuvant and topical induction with a neat preparation of the test article (20.2% PHMB). The sensitisation response of the animals was determined 24 and 48 hours after challenge by assessing the degree of erythema.

Following challenge with the neat test sample (20.2% PHMB), scattered mild redness or moderate diffuse redness was observed in the 18/20 of test animals at 24h and 16/20 at 48h (average scores of 1.4 at 24h and 1.2 at 48h). Scattered mild redness was observed in 4/10 of the control animals at 24h and 2/10 at 48h. The net frequency of response at 24h was 50%. Under this test condition, PHMB should be considered as a strong sensitiser.

Following challenge with a 6% (w/v) PHMB in deionised water, scattered mild redness or moderate diffuse redness was observed in 5/20 test animals at 24h and 2/20 at 48h (average scores of 0.3 at 24h and 0.1 at 48h) and scattered mild redness was observed only in 1 of the ten control animals at 24h. The net frequency of response at 24h was 15%. Under this test condition, PHMB should not be considered as a skin sensitiser according to classification criteria.

In the positive control group, challenge of previously induced guinea pigs with a 3% w/v preparation of 2-mercaptobenzothiazole elicited a strong skin sensitisation response.

In **Jackson, 1980b**, a test according to Magnusson and Kligman was performed on an aqueous solution of 20% PHMB. The protocol was consistent with OECD guideline 406 except that no SLS was applied during induction although no signs of irritation during induction were reported. Intradermal induction was performed with 0.2% PHMB in deionised water and Freund's complete

adjuvant and topical induction with a neat preparation of the test article (20.2% PHMB). Challenge was performed with the neat test sample (20.2% PHMB).

Challenge of test and control guinea pigs resulted in signs of mild to moderate erythema in 14 out of 20 test animals and mild erythema in 1 out of 8 controls at 24 hours (net frequency of response of 57.5%). At 48 hours mild to moderate erythema was present in 15 out of 20 test animals and mild erythema was still present in 1 control animal (net frequency of response of 62.5%). Although 1 control showed signs of skin irritation, the test material should be considered as having caused moderate to strong skin sensitisation under the conditions of this study.

Jackson, 1980b also evaluated the sensitising potential of PHMB in a Buehler test. The protocol was consistent with OECD guideline 406 except that only 10 animals were included in the treated group whereas 20 are recommended and induction procedure was repeated 10 times over 3 weeks. Induction and challenge were performed with 2% PHMB in water and re-challenge was performed with aqueous solutions of 4, 2 or 0.2% PHMB.

Challenge resulted in signs of faint erythema in 6 out of 10 test animals at 48 hours but there were no signs of erythema in any of the control animals (net frequency of response of 60%; strong potency). Rechallenge with a 4% solution of PHMB resulted in faint to moderate erythema in 8 out of 9 test animals and 3 out of 10 controls (net frequency of response of 59%; moderate potency). Rechallenge with a 2% solution of PHMB resulted in faint erythema in 3 out of 10 test animals, but not in controls (net frequency of response of 30%; moderate potency). Rechallenge with a 0.2% solution of PHMB did not cause an erytematous response in either test or control animals. In conclusion, a 2% solution of PHMB is a moderate to strong sensitiser to guinea pig skin under the conditions of the study.

In a further study (**Jackson *et al.*, 1983a**) the effect of variation in induction concentration of ¨PHMB on skin sensitisation in the guinea pig was studied according to the Buehler method. The protocol was consistent with OECD guideline 406 except that only 10 animals were included in the treated groups whereas 20 are recommended and that induction procedure was repeated 10 times over 3 weeks.

Main results in the various test conditions were as reported in Table 9 below.

Table 9: Response to PHMB in Jackson *et al.*, 1983a

Induction Conc.	Challenge Conc.	Re-challenge conc.	Response in test animals	Response in control animals	Net response	Potency
0.3%	0.3% - 0.15% - 0.075% - 0.03%	-	0/10	0/10	0%	Not sensitising
0.8%	0.8% - 0.4% - 0.2% - 0.08%	-	0/10	0/10	0%	Not sensitising
1.3%	1.3% - 0.65% - 0.325% - 0.13%	-	0/10	0/10	0%	Not sensitising
1.8%	1.8% - 0.9% - 0.45% - 0.18 %	-	0/10	0/10	0%	Not sensitising
2.0%	2.0%	- 2.0%	1/10 0/10	0/10 0/10	10% 0%	Not sensitising Not sensitising

		15%	6/10	0/10	60%	Strong
1.2%	1.2%	- 1.2% 15%	0/10 0/10 3/10	0/10 0/10 0/10	0% 0% 30%	Not sensitising Not sensitising Moderate
5%	15%	- 2% 1.2%	6/8 6/8 4/8	0/10 0/10 0/10	75% 75% 50%	Strong Strong Moderate

It was concluded that PHMB is a moderate to strong sensitiser to the skin of guinea pigs above induction concentration of 1.2% under the conditions of this study.

Jackson, 1983b also investigated possible cross-reactivity with chlorohexidine gluconate in a study performed according to Magnusson and Kligman method. The protocol was consistent with OECD guideline 406 except that no SLS was applied during induction although no signs of irritation during induction were reported. Intradermal induction was performed with 0.25% PHMB in water and topical induction with 20% PHMB. Challenge was performed with 20% PHMB or various concentration of chlorohexidine gluconate.

Challenge of test and control guinea pigs with 20% PHMB resulted in signs of irritation in 8 out of 20 test animals and in 3 out of 8 control animals (net frequency of response of 2.5%). No cross-reactivity with chlorohexidine gluconate was observed. Re-challenge with 20% PHMB resulted in response in 3 out of 20 test animals (net frequency of response of 15%). Under the conditions of the study PHMB was not considered to be a skin sensitiser according to classification criteria.

Another Guinea Pig Maximisation Test was performed according to guideline OECD 406 and GLP (**Richeux, 2002c**) on 20% aqueous PHMB diluted in physiological saline. Intradermal induction was performed with 0.15% PHMB and topical induction with 20% PHMB. Challenge was performed with 20% or 10% PHMB.

24 hours after challenge moderate erythema was observed in one animal out of 10 at the 20% challenge treatment site (net frequency of response of 10%) and for one animal out of 10 at the 10% concentration site in the test group (net frequency of response of 10%). No reactions were evident in the control group. 48 hours after challenge moderate erythema was observed in one animal at the 10% treatment site in the test group (net frequency of response of 10%). No reactions were evident in the control group. Under the conditions of this study, PHMB is not considered as a dermal sensitiser according to classification criteria.

5.4.1.2 Human data

Two studies have been conducted using human volunteers to assess the potential for skin sensitisation of PHMB.

In the first (**Hink, 1976**) the capacity of PHMB to cause light induced dermal toxicity was assessed in a human volunteer study using an adaptation of the repeat insult patch test (RIPT)procedure of Draize. A group of 26 volunteers were exposed to a 20% aqueous solution of PHMB diluted further in water (1% v/v PHMB) dosed three times per week for 3 or 4 consecutive weeks (giving a total

number of applications of 9 or 12). Exposure was by means of a skin patch moistened with 0.4ml of test solution applied to the upper arm. To increase the skin penetrating properties of the sample, sodium lauryl sulphate was added to the solutions to provide a final concentration of 0.01% in the patch solutions. The patches were applied at noon and removed 24 hours after application. Immediately after patch removal the test site was exposed to natural sunlight for one hour. Additionally, a challenge application was made 6 weeks after the initial exposure and the skin assessed 48 and 96 hours following application.

During induction there was no evidence of skin irritation except for 1 individual who had a definite erythema following the 4th and 5th applications and minimal erythema following the 3rd and the 6th through the 12th application. No reaction was observed further to challenge exposure and under the conditions of this study PHMD did not elicit sensitisation at a topical dose of 1%.

In a second study (**Smith, 1981**) the human skin sensitisation potential of dilutions of PHMB has been assessed in a total of 191 human subjects over 3 panels. Volunteers were exposed to aqueous solutions of PHMB three times per week for a total number of applications of 10. Exposure was by means of a skin patch moistened with 0.5ml of test solution applied to the upper arm.

A preliminary panel of 49 subjects was exposed during induction at 2% PHMB for the 6 first patches. Due to low level of irritation, exposure level was increased to 4% for the 3 next patches. On the appearance of symptoms of skin sensitisation in a number of subjects, the concentration was reduced to 2% for the remainder of the induction patches of this panel. At challenge, 8 subjects out 49 (16%) elicited skin reactions at 2% PHMB, 7 out of 49 (14%) at 1% and 0.5% PHMB and 2 (4%) subjects gave weak reactions at 0.1% PHMB.

In the main panel 114 subjects were exposed to 4% PHMB during induction as a first result of the irritation level from the preliminary panel. As the number of cases of reactions increased in the preliminary panel, the concentration was decreased to 2% from the 4th patch until the end of the induction phase. At challenge the concentrations chosen were 0.5%, 0.2%, 0.1% and 0.05% in view of the result of the preliminary panel. 18 subjects out 114 (16%) elicited skin reactions at 0.5% PHMB, 7 out of 114 (6%) at 0.2% PHMB and none at 0.1% and 0.05% PHMB. The intensity of the reactions was generally lower than that observed from the preliminary panel. Two other panellists had reactions which appeared during the 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.

In an additional panel 28 subjects were exposed to 2% PHMB during induction for 5 patch exposures before the results of the preliminary panel indicated this was a sensitising level. Therefore the remaining patches were applied as distilled water applications to avoid unnecessary risk. The concentrations chosen for challenge were 0.5%, 0.2%, 0.1% and 0.05%. 1 subject out of 18 reacted (5%) to the high level of 0.5% only. All other subjects gave negative results.

Overall, the results showed that PHMB is capable of causing skin sensitisation in human if repeated lengthy exposure is made at concentrations of at least 2% PHMB.

In over 30 years of manufacturing PHMB at sites in both the UK and the USA, no cases of dermatological problems have been reported to the occupational health unit at either site. It is however recognized that appropriate precautions are taken by the workforce to ensure that exposure is minimized.

PHMB has been used for many years as a water disinfectant in the treatment of swimming pools. More recent applications include use as an antimicrobial or preservative in a wide range of medicinal and cosmetic products and PHMB is listed in Annex VI of the European Cosmetics Directive 76/768/EEC as a preservative, with a maximum allowed use concentration of 0.3%.

Three patch test studies on PHMB were identified in the literature.

In the article of **Schnuch (2000)** a population of 1554 patients suspected as having contact allergies to cosmetics and medicaments were exposed to PHMB at 2.5% in aqueous solution. 389 patients were exposed for 1 day and 1165 for 2 days. On day 3, 6 patients (0.4%) had a positive reaction (+). One of the reactions may have been false-positive.

In the article of **Schnuch (2007),** of a total of 1975 patients examined, 10 patients (0.5%) had a positive reaction to PHMB at 2.5% and 16 patients (0.8%) to PHMB at 5%. However, probably at least 4 reactions at 2.5% may be interpreted as doubtful or irritant, i.e. false positive, as they were not confirmed by simultaneous reactions to higher concentrations. Some cases (4) with stronger reactions (++) corroborate that PHMB is a sensitiser albeit a rare one.

In an earlier study (**McFadden, 1998**) 2 out of 374 patients (0.5%) reacted positively to PHMB patch tested at 2.5% PHMB in water.

5.4.2 Respiratory system

No data

5.4.3 Summary and discussion of sensitisation

Animal data are summarised in Table 10 below.

Table 10: Summary of skin sensitisation studies in animals on PHMB

Species	Method	Induction concentration	Challenge concentration and net frequency of response	Result	Reference
Guinea pig	Maximi-sation test OECD 406	ID: 0.06% Topical: 20%	6% PHMB: 15% 20% PHMB: 50%	6% PHMB: not sensitising 20% PHMB: sensitising; strong potency	Duerden, 1993
Guinea pig	Maximi-sation test	ID: 0.2% Topical: 20%	20% PHMB: 62.5%	Sensitising; strong potency	Jackson, 1980b
Guinea pig	Buehler test	Topical: 2%	2% PHMB: 60% Rechallenge 4%: 59% Rechallenge 2%: 30% Rechallenge 0.2%: 0%	Sensitising; strong potency Sensitising; moderate potency Sensitising; moderate potency Not sensitising	Jackson, 1980b

Guinea pig	Buehler test	Topical: 0.3-5%	0.03-15% PHMB (see Table 9)	Sensitising. Moderate to strong reaction were observed at concentration of induction ≥ 1.2% and challenge ≥ 15% or at concentration of induction ≥ 5% and challenge ≥ 1.2%.	Jackson, 1983a
Guinea pig	Maximi-sation test	ID: 0.25% Topical: 20%	20% PHMB: 2.5% Rechallenge 20%: 15%	Not sensitising.	Jackson, 1983b
Guinea pig	Maximi-sation test OECD 406	ID: 0.15% Topical: 20%	20% PHMB: 10% 10% PHMB: 10%	Not sensitising.	Richeux, 2002c

ID: intradermal

Several studies investigated skin sensitising potential of PHMB in guinea pigs. In maximisation tests, whereas some tests report mild response consistent with an absence of classification for skin sensitisation, PHMB induced moderate to strong responses in other maximisation studies. The discrepancy in these results can not be entirely explained by the level of exposure to PHMB. Sensitisation was also observed in Buehler test with repeated inductions. Investigation of dose response showed that responses of moderate to strong potency were induced from induction concentration of 1.2% PHMB in these studies.

Overall, the positive responses observed in several studies indicate that PHMB is a skin sensitiser in animals.

Human data are summarised in Table 11 below.

Table 11: Summary of human studies on skin sensitisation of PHMB

Method	Number subjects sensitised / total number of subjects	Result	Reference
RIPT	0/26	Volunteers were exposed to a 24h-exposure to 1% PHMB in water and a 1h-exposure to sunlight for 9 to 12 times over 3 or 4 weeks. At week 6, they were challenged with 1% PHMB. No reaction was observed.	Hink, 1976
RIPT	Preliminary: 8/49 (16%) Main: 18/114 (16%) Additional: 1/28 (3%)	Volunteers were exposed to 2 and/or 4% PHMB for 24h for 10 times over 4 weeks. At week 6, they were challenged with concentration ranging from 0.05 to 2% PHMB. Positive reactions were observed in all panels above certain levels of challenge concentrations.	Smith, 1981
Patch test	2.5% PHMB: 6/1554 (0.4%)	Patients suspected as having contact allergies to cosmetics and medicaments were exposed to PHMB at 2.5% in aqueous solution. 6 patients (0.4%) had a positive reaction.	Schnuch, 2000
Patch test	2.5% PHMB: 10/1975 (0.5%) 5% PHMB: 16/1975 (0.8%)	Patients were exposed to PHMB at 2.5% and 5% in aqueous solution. Respectively 10 and 16 patients had a positive reaction.	Schnuch, 2007
Patch test	2.5% PHMB: 2/347 (0.5%)	Patients were exposed to PHMB at 2.5% in aqueous solution. 2 patients had a positive reaction.	McFadden, 1998

Although no sensitisation was observed in RIPT in volunteers exposed to 1% PHMB, repeated lengthy exposure to PHMB from 2% caused a significant level of sensitisation.

Patch tests on patients report sensitisation to PHMB at a very low frequency (0.4 to 0.8%) which are considered as a positive outcome of the study. However, it should be noted that PHMB is usually used at low concentrations in consumer products and this may explain the observation that PHMB is a rare contact sensitiser in humans.

Overall, PHMB is a skin sensitiser in guinea pigs and human studies indicate that PHMB is a skin sensitiser in humans, although with a rare frequency of sensitisation in the current conditions of consumer uses. Classification **R43** is therefore warranted (CLP Skin Sens. 1 – H317).

Relatively low incidences from human data support classification as CLP Skin Sens 1B-H317 according to the 2nd ATP to CLP Regulation.

In animal studies identifying skin sensitisation, moderate to strong potency was observed. However, in the light of the discrepancy seen in the various animal test results, overall potency of PHMB is difficult to evaluate. The positive Buehler tests were performed with repeated phases of induction and this deviation makes the result difficult to compare to potency guidelines for Buehler test. Besides, results from maximisation test by Jackson 1980b were at borderline between moderate and strong potency categories. Only the maximisation test by Duerden 93 clearly indicates a strong potency but was not consistent with results of Richeux 2002c. On the basis of these uncertainties,

no specific concentration limits are proposed. Besides, no guideline for setting specific concentration limits based on human data is available at the European level.

Highest response rates (>60% in Jackson et al., 1983a) may support subcategory 1A. However taking low incidences in humans and results from all animal studies into account, CLP Skin Sens 1B –H317 is considered as adequate.

5.5 Repeated dose toxicity

5.5.1 Repeated dose toxicity: oral

A summary of the main results on repeated dose toxicity of PHMB by oral route is provided in Table 12. The absence of serious health effects in the rat study and at doses ≤25 mg/kg (corresponding to the upper guidance value of 100 mg/kg for a 90 day study) in the 12 month study in dogs suggest the conclusion that no classification is proposed for this route.

Table 12: Summary of Repeated Dose Toxicity by oral route

Duration	Species Strain Sex no/group	Dose levels	Results	LO(A)EL	NO(A)EL	Reference
				[mg/kg bw/day]		
1 year	Dog; Beagle; Males/ Females; 4/sex	Administration in the diet at concentrations of 300, 1500, 4500 (reduced to 3000 during week 11 or 12) ppm for both sexes. The maximum estimation of doses levels are, respectively, 11, 54, 108 (3000 ppm) and 169 (4500 ppm) mg/kg/j.	Severe signs of toxicity were noted at 4500 ppm which necessitated sacrifice of 3 males at this concentration. As a result the top concentration was reduced to 3000 ppm. Only minor changes in clinical chemistry were observed at the low dose. Treatment-related histopathological findings were present in the following organs of animals that received 4500/3000 ppm: - skin: dermatitis of the scrotum in two males killed intercurrently, dermatitis of the chin and limbs in one female killed intercurrently and slight dermal mononuclear cell infiltration and acanthosis of the scrotal skin in one male killed intercurrently, - liver : eosinophilic introcytoplasmic inclusion bodies in all animals with minimal single cell necrosis in the liver in one male killed intercurrently and minimal to slight cellular swelling in all animals killed intercurrently and in one female at termination, - kidney : tubular hyaline droplet formation in three males killed intercurrently, - testis : slight bilateral testicular tubular degeneration in one male killed intercurrently and marked bilateral testicular tubular degeneration accompanied by moderate Leydig cell hyperplasia in one male at termination.	108	54	Horner, 1995
104 weeks	Rat; Alpk:AP_f SD Males/ Females 64/sex	Administration in the diet at concentrations of 0, 200, 600 or 2000 ppm, corresponding to dose levels of 12, 36 or 126 mg/kg and 15, 45 or 162 mg/kg in males and females, respectively	No treatment related clinical signs, opthalmoscopic findings or effects on any haematological or urinalysis parameter. Slightly raised plasma alkaline phosphatase, predominantly in females at 2000 ppm and slight increased incidence of hepatocyte fat and spongiosis in males at 2000 ppm	126 – 162	36 - 45	Horner, 1996

5.5.2 Repeated dose toxicity: inhalation

A study was conducted to determine the toxicity of PHMB to rats from administration via nose-only inhalation for a period of 28 days and was performed according to GLP and guideline OECD 412 (**Noakes, 2006**). Groups of 5 male and 5 female rats were exposed for 6 hours per day, 5 days per week for 28 days to 0.0239 (MMAD range – 0.32-1.30 µm), 0.257 (MMAD range – 1.70-4.03 µm), or 2.47 µg PHMB/l (MMAD range – 1.88-2.40 µm) prepared from aqueous solution of PHMB 20%. Additional groups of 5 animals/sex exposed to 0, 0.0239 or 2.47 µg/l were retained without treatment for a further 13 weeks in the recovery phase. Clinical observations were made twice daily on exposure days, once daily on non-exposure days and then weekly during the recovery period. Bodyweights were measured weekly and food consumption was measured continuously throughout the study. At the end of the scheduled period, the animals were killed and examined for post mortem. Cardiac blood samples were taken for clinical pathology from all animals, selected organs were weighed and specified tissues were taken for subsequent histopathological examination. The analysed concentrations of PHMB were 0.0239 (MMAD range – 0.32-1.30 µm), 0.257 (MMAD range – 0.48-5.06 µm), and 2.47 (MMAD range – 0.67-1.67 µm) µg/l for the low, mid, and high dose group, respectively.

There were no deaths attributable to treatment. There were no clinical signs that were attributable to exposure to PHMB at up to 2.47µg/l. Clinical observations during the exposure periods and post-exposure were typical of those associated with the restraint of the animals for a nose-only exposure. Bodyweights were lower than for the controls for males exposed to 0.257 µg/l or 2.47 µg/l. There was some evidence of recovery in bodyweight, following cessation of exposure for males at 2.47µg/l. Food consumption was slightly low in weeks 2 and 4 for males exposed to 0.257 and 2.47 µg/l. There were no changes in haematology or blood clinical chemistry parameters that were of toxicological significance.

Lung weights were slightly high for males and females exposed to 2.47 µg/l and thymus weights slightly high for males only at this exposure concentration. No macroscopic treatment-related findings were observed at the examination post mortem.

Treatment-related microscopic findings were recorded in the larynx, trachea and lungs. On completion of the 28 day exposure period, squamous metaplasia was seen in the larynx of males and females at 0.257 and 2.47 µg/l, and tracheal inflammation for males and females at 2.47 µg/l. No similar findings were present 13 weeks following cessation of treatment for animals previously exposed to 2.47 µg/l. Pneumonitis and bronchitis in the lung were seen for males and females exposed to 2.47 µg/l, both at end of the exposure period and at the end of the recovery period. However, the pneumonitis was observed to be slightly reduced in severity at the end of the recovery period. Since the pneumonitis and bronchitis were only observed at the high concentration, it is judged to be the result of a primary irritant response.

The higher thymus weight for males only exposed to 2.47µg/l, in the absence of any histopathological changes, was considered to be of unknown toxicological significance. Based on the transient histopathological changes in the larynx and trachea observed at the mid and high dose, some bodyweight changes at these exposure concentrations and some non-resolving histopathological changes in the lungs at the high dose, the clear NOAEL was considered to be 0.0239µg PHMB/l for both systemic and local effects on the respiratory tract.

In another 28-day inhalation study (**Carney, 1976**) rats (4/sex/group) were exposed to atmospheres containing respirable particles of PHMB (prepared from aqueous solution of PHMB 20%; mean diameter not specified) at concentrations of 26, 12.5, 2.75, 0.25 and 0.025 µg/L PHMB for 6 hours per day for 5 days a week, for three weeks, snout-only. The study was performed before adoption of guidelines and its interpretation was limited by poor reporting. Differences with the actual guidelines were noted: lower number of animals (5/sex/group required in guidelines), no information on monitoring of atmosphere, housing conditions and extent of haematological examinations, limited biochemical analysis and organs for histological examination.

I. 26 µg/L of PHMB - Exposure of rats to this concentration resulted in very severe nasal irritation and marked dyspnoea. The rats were exposed for only 6 hours and all animals died during the night following this exposure.

II. 12.5 µg/L of PHMB - Exposure of rats to this concentration also resulted in severe nasal irritation and dyspnoea. During the first three days of exposure all animals lost weight and their intake of food and water was very low. One female rat died towards the end of the fourth exposure and the remainder died overnight.

III. 2.75 µg/L of PHMB - The rats that were exposed to this concentration presented similar evidence of nasal irritation and dyspnoea, although less severe than that observed with 12.5 mg/m^3 (II above). Most of the animals in the test groups failed to gain body weight during the first three exposures. Some slight increase was recorded over the weekend (two treatment free days following the initial three exposures), however there was a dramatic weight loss in test animals after the fourth exposure. Food and water intake after the fifth exposure was practically nil. One male died during the sixth exposure. Two males and one female died overnight. The remaining male and three females were killed by Fluothane BP overdose. Blood samples taken for haematological examination revealed haemoconcentration in all animals and significant increases of methaemoglobin in all animals (9% in the male and 10, 5 and 5% in the three females). A low percentage of normoblasts were observed in one female animal and an increased number of neutrophils in another. No Heinz bodies were reported but it is not known whether they were investigated. Blood taken for biochemical analysis revealed no abnormalities. Histopathological examination of tissues revealed a moderate to severe pneumonitis in PHMB exposed animals. The reaction was patchy in character involving some alveoli and terminal bronchioles with more generalised macrophage activity throughout the whole of the alveolar bed. Small areas of epithelial desquamation were observed. Loss of cilia was also seen in certain areas of the tracheal epithelium. The thymus glands from all PHMB exposed animals showed severe depletion of lymphocytes and loss of normal architecture. There was a reduction in thickness of the cortex and a corresponding increase in reticular tissue. One female rat showed evidence of unilateral pyelonephritis.

IV. 0.25 µg/L of PHMB - Exposure of animals to this concentration resulted in moderate nasal irritation and tachypnoea. The animals failed to gain normal body weight and three males and two females actually lost weight over the thirteen exposure periods (one male died after this exposure). The experiment was terminated after the thirteenth exposure. Food consumption in male treated rats was low throughout. Urine taken directly after the last exposure revealed no abnormalities apart from a low output of urine from the treated males. The remaining animals were killed by Fluothane BP overdose. Blood taken for haematological examination again revealed significant amounts of methaemoglobin in all animals (5, 4 and 4% in males and 3, 7, 5 and 3% in females) and haemoconcentration. No other anomalies of the blood cells were reported. Biochemical analysis of the blood revealed no abnormalities. Histopathological examination of stained sections revealed slight to moderately severe pneumonitis. There was also evidence of accompanying resolution of the lung lesions in all the affected animals. No further information on this effect is provided in the study report and it is supposed that it refers to apparition of regenerative tissues (such as

hyperplasia) and/or fibrosis. The thymuses of 3 male and 3 female rats from the test group showed reduction in the cortical thickness and depletion of lymphocytes. Patchy loss of cilia in the tracheal epithelium was observed in three animals. The testis of one male showed degeneration of a few seminiferous tubules.

V. 0.025 µg/L of PHMB - Exposure to this concentration did not result in any signs of toxicity. Increases in body weight were erratic and low but intake of food and water was normal when compared with non-exposed control rats. No abnormalities were found in blood taken 18 hours after cessation of exposure. Urinalysis revealed no abnormalities. There was no evidence of either local or systemic chemical toxicity from histopathology.

5.5.3 Repeated dose toxicity: dermal

A summary of the main results on repeated dose toxicity of PHMB by dermal route is provided in Table 13. No classification is proposed for this route due to the lack of serious health effects ≤ 60 mg/kg/day (guidance value for dermal 28-day studies).

Table 13: Summary of PHMB repeated dose toxicity studies by dermal route

Duration	**Species Strain Sex no/group**	**Dose levels**	**Results**	**LO(A)EL**	**NO(A)EL**	**Reference**
				[mg/kg bw/day]		
21 days	Rat; Alpk:AP_f SD Males/ Females 5/sex	0, 20, 60, or 200 mg/kg	No clinical effects at any dose. Slight to moderate irritation of the skin in direct relationship to dose. No mortality at any dose. Apart from scabbing and erythema at the site of contact, there were no macroscopic findings. Histopathological changes were noted only at the site of contact and consisted in irritation and inflammation.	60 mg/kg for local irritation at the site of contact; the LOAEL was not established for systemic toxicity	200 mg/kg for systemic toxicity; 20 mg/kg (equivalent to 0.122 to 0.148 mg/cm² in males and 0.104 to 0.116 mg/cm² in females) for local irritation at the site of contact	Lees, 1993

5.5.4 Summary and discussion of repeated dose toxicity:

No significant toxicity is induced by PHMB by oral route or by dermal route at doses relevant for classification.

Repeated dose toxicity of PHMB has been assessed by inhalation in two studies, which are summarised in Table 14.

Table 14: Summary of PHMB repeated dose toxicity studies by inhalation

Duration	Species Strain Sex no/group	Dose levels	Results	LO(A)EL	NO(A)EL	Reference
				[mg/kg bw/day]		
28 days	Rat; Alpk:AP_f SD Males/ Females 5/sex	0.0239, 0.257, or 2.47 µg/l	No clinical findings at any concentration. No mortality at any concentration. Slightly lower bodyweight gain in males at the mid and top concentration. Increased lung weight in males and females and thymus weight in males at the top concentration. Increased liver weight for males at the mid and top concentration. No macroscopic changes. Microscopic changes consisted of squamous metaplasia in the larynx in animals exposed to the mid and top concentrations and pneumonitis and bronchitis at the top concentration. Also, inflammation of the trachea was observed at the top concentration.	0.257 µg/l	0.0239 µg/l	**Noakes, 2006**
28 days	Rat; SPF albino Males/ Females 4/sex	0.025, 0.25, 2.75, 12.5 or 26 µg/l	In the high dose group, very severe nasal irritation and marked dyspnoea were noted ante-mortem, only a single exposure was possible and all treated rats died within 24 hours of first exposure. The concentration of 12.5 µg/L respirable particles proved particularly toxic. Severe nasal irritation and dyspnoea were evident and all rats died following the fourth exposure period. At lower concentrations, 2.75 or 0.25µg/L, moderate to severe eye and nasal irritation was seen with associated pneumonitis; body weight gains, food and water intakes were all reduced and methaemoglobin was evident. Mortality was also observed at 2.75 µg/L in 4 animals during or after the sixth exposure. The thymus glands from all PHMB exposed animals at 2.75 µg/L showed severe depletion of lymphocytes and loss of normal architecture. Haematological examination revealed haemoconcentration and significant increases of methaemoglobin for all animals exposed to 2.75 or 0.25 µg/L. At 0.25 µg/L, one male died after the 13 thirteenth exposure. Bodyweight gains at the low concentration, 0.025 µg/L, were low and erratic.	0.25 µg/l	0.025 µg/l	**Carney, 1976**

Repeated inhalation of PHMB caused severe irritation of the respiratory tract from 0.25µg/L and above in rats as evidenced by microscopic alterations such as squamous metaplasia in the larynx and pneumonitis and bronchitis. Moderate to severe pneumonitis with areas of epithelial desquamation was reported at 2.75 µg/l. In one study, the lung lesions were not reversible after a

long recovery period of 13 weeks. From 0.25µg/L, significant methemoglobinemia and effects on thymus were also observed. Although only an increase of thymus weight was observed in Noakes, 2006 at the highest dose of 2.47 µg/l, reduced lymphocytes and cortical reduction were seen from 0.25 µg/L and severe at 2.76 µg/l in Carney, 1976.

Repeated inhalation also resulted in mortality at 0.25µg/L and higher doses. Mortality occurred at the highest dose after a single exposure, which justify classification for acute inhalation toxicity. However, delayed mortalities were also observed after repeated exposure from doses two orders of magnitude lower, which justifies to consider it also for repeated toxicity classification.

On the basis of the severity of the effects caused by inhalation of PHMB (delayed mortality, thymus atrophy and to severe inflammatory and metaplastic changes in the respiratory tract), the absence of reversibility of inflammation in the respiratory tract and the very low doses causing these effects compared to the guidance value for Cat 1 (CLP) of 0.06 mg/l/6h (28-days), **classification T; R48/23 is warranted** (CLP STOT RE 1 - H 372).

By inhalation the primary target organ is the respiratory tract and no effect warranting classification are identified by oral and dermal route. It is therefore proposed to allocate to the hazard statement H372 the following additional statements: H372 (Causes damage to the respiratory tract through prolonged or repeated exposure by inhalation).

5.6 Mutagenicity

A summary of the main results on mutagenicity of PHMB is provided in Table 15 and 16. Based on the studies available PHMB is not considered mutagenic and no classification is proposed for this endpoint.

5.6.1 In vitro data

Table 15: Summary of genotoxicity of PHMB *in vitro*

Test system Method	Organism/ strain(s)	Concentrations	Result		Remark	Reference
			+ S9	- S9		
Salmonella typhimurium OECD 471	*Salmonella typhimurium* Strains TA 1535, 1537, 1538, 98, and 100	+S9: 0.063, 0.31, 1.57, 7.84, 39.2, or 98 µg/plate -S9: 0.063, 0.31, 1.57, 7.84, 39.2, or 98 µg/plate	Not mutagenic	Not mutagenic	Cytotoxicity +S9 – toxicity evident at 196 and 980 µg/plate -S9 – toxicity evident at 196 and 980 µg/plate	Callander, 1989
Chromo-somal aberration using human lymphocytes OECD 473	Human lymphocytes	+S9: Donor 1: 4.9, 19.6, and 36.8 µg /ml; Donor 2: 4.9, 9.8, and 49 µg /ml -S9: Donors 1 and 2: 0.98, 4.9, and 9.8 µg /ml	Not mutagenic	Not mutagenic	The mitotic index was significantly decreased from the negative control at the top concentration (± metabolic activation) in individuals 1 and 2. For donor 1 the mitotic index at 9.8 µg PHMB/ml was 44% and 25% of control ± metabolic activation, respectively. For donor 2 the mitotic index was 54% of control at 49 µg PHMB/ml with metabolic activation and 52% of control at 9.8 µg	Howard, 1989

					PHMB/ml without metabolic activation.	

5.6.2 In vivo data

Table 16: Summary of genotoxicity of PHMB *in vivo*

Type of test Method/ Guideline	**Species Strain Sex no. per group**	**Freq. of applic.**	**Sampling times**	**Dose levels** [mg/kg bw]	**Results**	**Remarks**	**Reference**
Mouse bone marrow micronucleus test OECD 474	Mouse; C57BL/6Jf CD-1/Alpk Male and Female 5/sex	Single dose	24, 48, and 72 hours after dosing	250 or 400 mg/kg	Under the conditions of this assay, PHMB does not produce micronucleated polychromatic erythrocytes and is therefore not clastogenic.	These dose levels represent 50% and 80% of the median lethal dose of 500 mg PHMB/kg based on lethality data.	Randall, 1989
Unscheduled DNA synthesis assay using rat liver; No specific regulatory guideline was available for this study at the time it was conducted, but it was conducted according to scientific standards acceptable at the time.	Rat; Alpk:AP_fSD Males; 2 animals/ group for the first experiment and 3 animals/ group for the second experiment.	Single dose	4 and 12 hours after dosing	147 or 294 mg/kg	Under the conditions of this test, PHMB did not produce damage to DNA, as measured by the UDS assay, in the rat liver.	Excessive salivation and a subdued attitude at the top dose. The positive control, 6-dimethylamino phenylazobenzthiazole (6BT), induced a significant increase in unscheduled DNA synthesis, thus confirming the ability and sensitivity of the test system to detect UDS.	Trueman, 1989

5.6.3 Human data

No data available

5.6.4 Summary and discussion of mutagenicity

PHMB is not considered mutagenic.

5.7 Carcinogenicity

After examination of the full study reports, in all, there have been three valid carcinogenicity studies carried out on PHMB – one oral study in the mouse, one oral study in the rat and a dermal study in the mouse.

5.7.1 Carcinogenicity: oral

Mouse/oral carcinogenicity study

In a life-time feeding study in the mouse (**Milburn, 1996**), conducted according to US EPA guideline 83-2, groups of 55 male and 55 female C57B1/10J$_f$CD-1 mice were fed diets containing 0, 400, 1200 or 4000 ppm PHMB for a period of 2-years. The mean received dose for the 400, 1200 and 4000 ppm groups were 55, 167 or 715 mg/kg/day in males and 69, 217 or 856 mg/kg/day in females, respectively. Animals were submitted to full post mortem examination and samples of organs were preserved for microscopic examination including anus and recto-anal junction that were added from week 70.

Administration of 4000 ppm PHMB was in excess of a maximum tolerated dose (MTD) based on lowered bodyweight, decreases in weight gain, increases in food consumption and utilisation (week 1-12), all starting early in the study. Bodyweights were up to 20% (males) and 15% (females) lower than those of concurrent controls in the second year of the study. In terms of bodyweight gain, there was a reduction of 35-42% (males) and 22-33% (females) compared to the controls during weeks 53-79.

Mortality was increased in females receiving 4000 ppm from week 26 throughout the study and in males receiving 4000 ppm PHMB from week 30 to week 90. A similar number of males in the control and 4000 ppm groups survived to 2 years. There was no adverse effect on survival in mice receiving 1200 or 400 ppm PHMB. Haemangiosarcoma was the most frequent factor contributing to the death of mice given 4000 ppm PHMB (see cause of death in Table 17).

Increased food consumption (from week 10 and throughout the remaining study) and less efficient food utilisation (than controls) was noted for both sexes at this dose level. The main treatment related clinical observation in males and females at the top concentration was anal swelling. The first noted occurrence was in week 18 for males and week 53 for females. An increased incidence of irregular breathing was observed in males and females receiving 4000 ppm PHMB. In all groups of treated females there was an increased incidence of abnormal respiratory noise which was not correlated by histopathologically abnormal findings. There was an increased number of male mice receiving 4000 ppm PHMB with discharge from the eye. There was also an increase in the number of mice with torn ears and of those with hairloss in the 400 and 1200 ppm dose groups.

At termination, there was an increase in haemoglobin, haematocrit and red cell count in both sexes receiving 4000 ppm and in females receiving 1200 ppm PHMB. The effect was thought to be related to slight dehydration.

In mice receiving PHMB a variety of treatment-related non-neoplastic changes was seen in the liver, gall bladder and recto-anal junction:

Non-neoplastic lesions

Liver

There were treatment-related changes in the livers of all dosed animals although no marked difference of incidence was seen at 400 ppm. Hepatocyte hypertrophy and increased ploidy were observed in both sexes at all dose levels with the incidence increasing with increased dose level and no corresponding effects on liver weights. The incidence at 4000 ppm was approximately 50% in both sexes. However, there was only a single incidence in both sexes at 400 ppm. There was increased pigmentation (lipofuscin and haemosiderin, no data given on proof by special staining) in the livers of males at 4000 ppm and females at all dose levels although at 400 ppm there was only a single incidence in a female. None of these changes was seen in control animals. There was an

increased incidence of hepatitis which was mainly of minimal severity in males and females given 4000 ppm PHMB and females given 1200 ppm PHMB compared with controls. The incidence of increased extramedullary haematopoiesis was increased in both sexes given 4000 ppm PHMB compared with controls.

Non-neoplastic lesion in the liver	Males				Females			
Dose Group (ppm of PHMB)	0	400	1200	4000	0	400	1200	4000
No. of. livers examined	55	55	55	55	55	55	55	55
Hepatocyte hypertrophy Minimal-moderate	0	1	7	29	0	1	19	27
Hepatocyte ploidy minimal-moderate	0	1	7	29	0	1	20	21
Pigmentation minimal-slight	0	0	0	20	0	1	6	21
Hepatitis minimal	4	3	5	15	8	8	13	15
Extramedullary haemopoiesis miminal-marked	4	8	4	12	3	6	5	10

Gall bladder

Luminal dilatation in the gall bladder was increased in both sexes at 4000 ppm compared with controls. This change had been noted at necropsy and the lesion was of marked/moderate severity. There was a dose related increase in epithelial hyperplasia in males and females at 1200 and 4000 ppm. One male at 400 ppm had slight epithelial hyperplasia and casts in the lumen. Epithelial hyperplasia was not observed in controls.

Non-neoplastic lesions in the gall bladder	Males				Females			
Dose Group (ppm of PHMB)	0	400	1200	4000	0	400	1200	4000
No. of gall bladders examined	45	43	45	48	53	51	53	45
Luminal dilatation (moderate/marked)	9 (9/0)	3 (3/0)	6 (6/0)	23 (11/12)	1 (1/0)	2 (2/0)	5 (5/0)	18 (6/12)
Epithelial hyperplasia (mimimal/slight/mmoderate)	0	1 (0/1/0)	2 (1/1(0)	12 (1/6/5)	0	0	3 (1/2/0)	6 (1/1/4)

Spleen

There was an increase in extramedullary haemopoiesis in the spleen of both sexes given 4000 pppm PHMB without any clear dose-relationship at other doses.

Non-neoplastic lesions in the spleen	Males				Females			
Dose Group (ppm of PHMB)	0	400	1200	4000	0	400	1200	4000
No. of spleen examined	55	55	55	55	55	55	55	55
Extramedullary haemopoiesis (slight/moderate/marked)	9 (4/2/3)	7 (1/5/1)	5 (1/2/2)	19 (5/9/5)	17 (11/5/1)	14 (6/5/3)	14 (4/7/3)	24 (6/9/9)

Recto-anal junction

The single mass and thickened/discoloured/prolapsed anuses recorded in high dose animals in necropsy correlated microscopically with treatment related changes around the recto-anal junction. These changes were of an inflammatory nature, mainly an inflammatory infiltrate with squamous epithelial hyperplasia and squamous metaplasia of the rectal glands in addition in some animals. There was an increased microscopical incidence of inflammatory change in males at all dose levels and females at 1200 and 4000 ppm compared with controls. The incidence was app. 81% in high dose males compared with 2% in male controls and 74%in high dose females compared with 20% in control females. Squamous metaplasia of the rectum at the recto-anal junction was increased in males given 4000 ppm PHMB compared with controls and there was a single incidence of hyperplasia of the rectum in a male at this dose level.

Non-neoplastic lesions in the recto-anal junction	Males				Females			
Dose Group (ppm of PHMB)	0	400	1200	4000	0	400	1200	4000
No. of recto-anal junctions examined	45	45	45	49	48	45	47	39
Inflammation (mimimal/slight/moderate/marked	1 (1/0/0/0)	10 (10/0/0/0)	20 (16/4/0/0)	40 (13/15/8/4)	10 (10/0/0/0)	4 (4/0/0/0)	22 (18/3/1/0)	29 (7/15/5/2)
Squamous epithelia hyperplasia	0	0	5	12	0	0	3	8
Squamous metaplasia rectal glands	0	0	1	7	0	0	1	2
Squamous metaplasia rectum	0	0	0	5	0	0	0	0
Hyperplasia rectum	0	0	0	1	0	0	0	0

There was a low incidence of change in the rectum proximal to the recto-anal junction. There was inflammation in three females and two males given 4000 ppm PHMB, in one male given 1200 ppm PHMB and minimal inflammation in one female given 400 ppm PHMB. Other changes in the rectum were necrosis in one male given 4000 ppm and hyperplasia in another male at this dose level. There was a low incidence of inflammation in the skin adjacent to the anus, which was minimally increased in males given 4000 ppm PHMB compared to controls. The prolapses in two males given 4000 ppm PHMB were confirmed microscopically and one female given 4000 ppm PHMB also had a rectal prolapse.

The anal swelling (and also discharge and sores) observed in several animals given 4000 ppm correlates with the findings of inflammation (observed in 1, 10, 20 and 40 males and in 10, 4, 22

and 29 females at 0, 400, 1200 and 4000 ppm, respectively; increased severity with increasing doses), squamous epithelial hyperplasia (observed in 0, 0, 5 and 12 males and in 0, 0, 3 and 8 females at 0, 400, 1200 and 4000 ppm, respectively) and squamous metaplasia (observed in 0, 0, 0 and 5 males at 0, 400, 1200 and 4000 ppm, respectively; not observed in females) in the recto-anal region. PHMB is poorly absorbed from the diet, and concentrates in the recto-anal region before evacuation. Consequently, the effects seen are attributable to chronic irritation caused by high concentrations of an irritant compound. This irritant effect (through biliary excretion) is also the likely cause of the two gall-bladder papillomas seen in males.

Skin

There was a minimal increase in the number of mice with reduced hair follicles in both sexes given 4000 ppm PHMB and in females given 1200 ppm PHMB which correlated with the hair loss recorded at necropsy.

Tumours

An altered tumour profile was present at 4000 ppm PHMB but the author considered significance of this change to be very uncertain in the presence of such marked toxicity. There was a decrease in the number of lymphosarcomas in both males and females. Pituitary gland adenomas were also decreased in female mice.

Conversely, there were increases in squamous cell carcinomas of the recto-anal junction in mice of both sexes (5 males and 8 females at 4000 ppm), one adenocarcinoma at the same site in a male from this group and a squamous cell carcinoma of the skin adjacent to the anus.

Neoplastic lesions in the recto-anal junction	Males				Females			
Dose Group (ppm of PHMB)	0	400	1200	4000	0	400	1200	4000
No. of recto-anal junctions examined	45	45	45	49	48	45	47	39
Squamous cell carcinoma	0	0	0	5 (10%)	0	0	0	8 (21%)
No. of ani examined	45	45	45	50	48	45	47	39
Squamous cell carcinoma	0	0	0	1 (2%)	0	0	0	0

Gall bladder papillomas occurred in two males (at 4000 ppm). None was seen in controls or in mice from lower dose groups.

Milburn (1966) reported that the highest incidence of treatment related tumours was in neoplasms of vascular origin, i.e. haemangiosarcomas, which are malignant tumours originating from vascular endothelium. In the C57BL/10JfCD-1/Alpk mouse this tumour type occurs relatively frequently in controls. The most frequent sites are liver, spleen, bone marrow, subcutaneous tissue, ovary and the tumour may occur at other sites. In individual mice tumours are often seen in more than one organ when the pattern of growth is consistent with a multi-centric origin rather than a single primary tumour with metastatic spread to other sites. Hence the numbers of mice bearing haemangiosarcomas, at any site, are considered to provide the most reliable basis for assessment.

Unlike it might be expected for multicentric vascular tumours other frequently affected sites such as spleen, bone marrow, subcutaneous tissue and ovary did not show treatment-related increases in tumour incidences in this mouse study. PHMB appears to mainly affect the presence of vascular tumours in the liver.

Dose-dependent increases in incidences of non-neoplastic lesions such as hepatocyte hypertrophy and ploidy and pigmentation were observed and could be interpreted to be related to tumour growth.

This type of vascular tumour is a common tumour in the C57 mouse, with published rates up to 14% reported. Internal historical-control incidences for haemangiosarcomas at any site, alone, from 1.8% to 18.3% (average 9.16%) in male mice and from 0% to 9.1% (average 4.42%) in female mice have also been reported at the test laboratory (Brown, 2002). This analysis did not contain separate data in control animal incidences in the liver.

In a peer review on all sections from liver and other slides in which proliferative vascular lesions were initially diagnosed by the study pathologist were re-examined and the reviewing pathologist did agree with the increase in vascular tumours but not always with diagnosis (haemangioma vs. haemangiosarcoma). A Pathology Working Group (PWG) consisting of a pathologist representing the company, the reviewing pathologist and three consultant pathologist was set up to examine those (coaded) slides with vascular lesions and to achieve a consensus diagnoses for each vascular tumours (Mann, 2002). Two other experts acted as chairperson and observer.

Following the PWG review, the consensus diagnosis for the evaluation of vascular tumours was as follows (Results from the original study of Milburn (1996) were added):

Table 17: Incidence of Animals with vascular tumours in the liver

	Dose Group (ppm of PHMB)			
	0	400	1200	4000
	Males			
Haemangiomas *Original-Total*	1/55 (2%) 0	1/55 (2%) 0	2/55 (4%) 1	8/55 (15%) 1
Haemangio-sarcomas *Original-Intercurrent* *- Cause of death* *Original-Total*	3/55 (5%) 4/29 4 4/55	0/55 2/26 0 2/55	6/55 (11%) 3/17 3 7/55	12/55 (22%) 16/31 14 20/55
Combined	4/55 (7%)	1/55 (2%)	8/55 (14%)	20/55 (36%)
	Females			
Haemangiomas *Orginal-Intercurrent* *0riginal-Total*	1/55 (2%) 0 0	0/55 0 0	2/55 (4%) 0 0	5/55 (9%) 0 0
Haemangio-sarcomas *Origin-Intercurrent* *- Cause of death* *Origin-Total*	0/55 0/30 0/55	0/55 0/36 0/55	2/55 (4%) 2/20 2 4/55	7/55 (13%) 9/38 8 13/55
Combined	1/55 (2%)	0/55	4/55 (7%)	12/55 (22%)

No statistical analysis available in the PWG report (Mann 2002) nor in the USEPA evaluation (USEPA 2003).

Table 18: Incidence of Animals with vascular tumours at any Site

	Dose Group (ppm of PHMB)			
	0	400	1200	4000
	Males			
Haemangiomas§	2/55 (4%)**	3/55 (5%)	4/55 (7%)	11/55 (20%)**
Haemangio-sarcomas *Origin-intercurrent#* *Origin-total#*	5/55 (9%)** 5/29 p0.002** 6/55 p0.001**	4/55 (7%) 4/26 6/55	6/55 (11%) 3/17 7/55	12/55 (22%)* 16/31* 20/55**
Combined	6/55 (11%)**	6/55 (11%)	9/55 (16%)	20/55 (36%)**
	Females			
Haemangiomas§	6/55 (11%)*	2/55 (4%)	5/55 (9%)	8/55 (15%)
Haemangio-sarcomas *Origin-intercurrent#* *Origin-total#*	6/55 (11%)* 3/25 p0.021* 7/55 p0.006**	4/55 (7%) 1/19 4/55	4/55 (7%) 2/20 5/55	10/55 (18%) 12/37 17/55*
Combined	8/55 (15%)**	5/55 (9%)	7/55 (13%)	15/55 (27%)*

No statistical analysis available in the PWG report (Mann 2002) .

Statistical analysis of vascular tumour rates at any sites according to Fisher's exact test and exact test trend was provided in the USEPA analysis (USEPA, 2003). Trend significance is denoted at control and pair-wise comparison with controls at the dose level. * $p<0.05$, ** $p<0.01$

Origin Original study report (Milburn, 1996), *intercurrent* (no of tumours/no. of deaths), *total* total no of test animals

Fisher's Exact Test, Trend test using Cochran-Armitage Test (Milburn, 1996)

$ No summary table available on the incidence of haemangiomas at any site in intercurrent deaths/killed animals and total no. of animals

Table 18a: Incidence of haemangiosarcomas at any sites/sites specified

	Males				Females			
Dose Group (ppm of PHMB)	0	400	1200	4000	0	400	1200	4000
Abdominal cavity	0/0$	0/1	0/1	0/0	1/1	0/1	0/1	0/0
Bone marrow	0/54	0/55	0/55	0/54	0/54	2/55	1/54	0/54
Limb	1/2	0/1	0/1	0/3	0/3	0/1	0/2	0/2
Liver	**4/55**	2/55	**7/55**	**20/55**	0/55	0/55	**4/55**	**13/55**
Ovary	-	-	-	-	1/54	0/54	1/53	1/54
Spleen	1/55	**3/55**	0/55	0/55	**5/55**	1/55	1/55	**3/55**
Subcut. tissue	0/55	1/55	0/54	0/55	0/55	2/55	0/55	0/55
Uterus	-	-	-	-	0/55	1/55	0/55	0/55

From Appendix K, Milburn,1996,: $ no. of findings/no. organs/tissues examined

Taking the original study and the PWG re-evaluation of selected slides into account it is apparent that for 'any site' the increase is statistically significant for haemangiomas and haemangiosarcomas, and haemangiomas/haemangioendotheliomas combined. At the mid and high dose level, increased incidences of vascular tumours at any site are mainly due to increases in vascular tumours in the liver (see Table 18a). Other sites (than liver) do not significantly contribute to incidences at all sites.

Although the PWG came up with slightly different numbers of vascular tumours, this observation will not significantly change.

Since no data from internal historical controls were available for the liver, a crude comparison to vascular tumours at any site would also reveal that liver tumours at the high dose level are clearly above mean and maximum values expected for internal controls.

Although no statistical analysis was presented, the incidences of haemangiomas and of haemangiosarcomas (and combined incidences thereof) in the liver of high dose male and female mice are clearly above control incidences. Although dossier submitter reported like Milburn did in his original report that 4000 ppm PHMB was a dose exceeding the MTD, high frequencies of haemangiosarcomas (e.g. 14 males of 31 intercurrent deaths died due to this tumour (Table 17)) that were identified as cause of death may question the significance of lower bodyweight in terms of indicating systemic toxicity.

While the study author did, PWG did not give interpretation on whether the high dose exceeds the MTD.

The 1200 ppm PHMB dose level was reported to achieve an appropriate maximum tolerated dose with reductions in bodyweight and non-neoplastic pathological changes in the liver (both sexes), changes of inflammatory nature in the recto-anal junction and changes in the gall bladder (females only). Bodyweights were 5-6% lower than those of controls during the second year of the study. In terms of bodyweight gain there was a reduction of 7-14% (male) and 5-10% (female) compared to controls during weeks 53-79 of the study. There was no effect on food consumption in mice receiving 1200 ppm PHMB.

Statistical analysis concluded that at 1200 ppm increases in vascular tumours at any site did not reach significance level (no stat. data available for the liver). However, when considering haemangiosarcomas in the liver more specifically, an increase was observed in males (11% vs. 5% in controls) (and in females (4%) vs. 0% in controls)). According to the PWG (Mann, 2002) the small difference in incidence of haemangiosarcomas in the liver between control and mid-dose males was considered a chance event because it did not attain statistical significance and approximates the historical control range of haemangiosarcomas at any sites (from 1.8% to 18.3%, with an average of 9.16%). Nevertheless, in the light of the clear increase of haemangiosarcomas in the liver at the high dose in males (22%), the smaller increase at mid-dose could be considered as treatment-related and biologically significant.

Mice receiving PHMB showed a reduced incidence of lymphosarcoma of the lympho-reticular system in comparison with controls which is considered to be treatment related (not an issue for PWG). Incidences of lymphosarcomas at any site were 30, 23, 13, 11 in males and 31, 26, 17 and 15 in females receiving 0, 400, 1200 or 4000 ppm PHMB. There were no other treatment related neoplastic changes.

In conclusion of this study a clear increased incidence of haemangiosarcomas (in the liver and any sites) is observed in males and females at 4000 ppm and a small increased incidence of haemangiosarcomas in the liver of male mice at 1200 ppm. Induction of squamous cell carcinomas in the recto-anal junction was considered related to chronic inflammation that was induced at the excessive top dose. Uncertainty remains about the low incidence of gall bladder papillomas at 4000 ppm, however increased incidences of epithelial hyperplasia interpretable as preneoplasias and evident from 1200 ppm onwards indicate that a potential could not completely be ruled out.

RAC considered it unlikely to explain higher rates of vascular tumours in the liver by chance and

concluded that data from the mouse carcinogenicity study of Milburn (1996) give some evidence of carcinogenicity.

In another mouse oral study (**Clapp et al., 1977a**) groups of 30 male and 60 female mice (Swiss-derived albino mice, strain not specified) were fed diets containing 0, 500, 1000 or 5000 ppm Baquacil SB, equivalent to 0, 100, 200 and 1000 ppm of the active ingredient PHMB, for one week prior to pairing and during mating. Feeding continued for the females throughout pregnancy and lactation. All offspring were weaned at 3 weeks of age. At 5 weeks of age 50 males and 50 females were selected from each group. The offspring were fed the same diets as the parents throughout the experiment. After a further 80 weeks 10 males and 10 females per group were killed for pathological examination. The experiment was terminated when the overall mortality had reached 80%, 97 weeks after selection of offspring.

Due to considerable evidence of fighting amongst male population mortalities in the males were high during the first 6 months of the experiment especially for controls and animals receiving 100 or 1000 ppm PHMB. (However, no data are available on the numbers affected. This was also the reason why the study was not considered to be acceptable by dossier submitter and for the biocide CAR). Mortalities in the females were generally low during the first 18 months of the experiments, and were lower on treated groups than on controls.

The male treated groups tended to have gained more weight than controls, apart from a lower weight gain in the first week. The weight gain of animals receiving 1000 mg PHMB was noticeably higher than controls between weeks 30-68 and at weeks 88 and 92 (not significant and for a number of time points not related to the dose).

It was reported that the female treated groups demonstrated very similar growth to controls up to about week 5. Between weeks 5 and 20 they all tended to grow less than controls, the differences from control being significantly different often enough during this period to suggest that this was a real difference for animals receiving 200 or 1000 ppm PHMB (however, no consistent dose relationship was seen). After week 20 these groups seem to have grown similarly to controls until the second year of the study. For both sexes the results from around one year onwards became very variable and the relative positions of the group means altered considerable over short periods. The only consistent effect for the whole treatment period (week 1-96) was a significant reduction in bodyweight gain and mean bodyweights compared to controls for females receiving 1000 ppm PHMB

After week 1 and up to at least week 20 mean food consumptions were very similar in male treated groups to control animals. During the following period animals receiving 200 or 1000 ppm PHMB consumed less than the other two groups. (However, food consumption in the 200 ppm group was lower than in the 1000 ppm group at half of the time intervals measured.) Treated females consumed consistently less food than controls certainly from week 4 onwards, and generally throughout the study. A number of times during this period food consumption was significantly lower for females receiving 200 and 1000 ppm PHMB (with a number of time intervals where food consumption was lowest for the 200 ppm group.) Lower food consumption was interpreted as being less palatable at least for the low and mid dose groups, while for the high dose females it was interpreted as indicative for a slight toxic effect.

There was increased absolute liver weight for males and females receiving 1000 ppm PHMB (significant for females only). (Significancy was not indicated in the table, no data on relative liver weight available.) There were no treatment related (non-neoplastic or neoplastic) increases in

histopathologic findings. However, with respect to vascular tumours of concern there are a number of treated animals bearing haemangiomas or haemangiosarcomas in the liver or at other sites.

Among mortalities haemangiosarcomas were reported in the liver of 1/29 male and in 1/30 female of the 1000 ppm group, 1 haemangioma in the mesenteric lymph nodes was reported in 1/24 males of the 200 ppm group. Another haemangioma was seen in the spleen of a female (1/29) of this group. Haemangiomas in the uterus were also found in 2/25 females at 100 ppm and 2/29 females at 200 ppm PHMB. No tumour of vascular origin was seen in control groups.

In the group of interim kills 1/10 males at 200 ppm had a haemangioma in the liver, a haemangioma was also seen 1/10 females at 200 ppm PHMB. No one was reported for the control animals of the interim kills at week 80.

At final kill at week 97 there was an haemangioma in the liver of 1/6 high dose females, 2/15 haemangiomas in the mesenteric lymph nodes of males at 200 ppm, and no vascular tumors in 4 males and 11 females of the control groups.

In laboratory historical controls of this strain 2 vascular tumours were reported in the liver of 118 males (1.7%) and 1 in 118 females (0.85%) in the years 1974/75.

In conclusion this study is of limited value due to the high fighting-related mortalities during the first 6 months, without any information about the numbers of animals affected. This means that it is unknown how many males were still alive at and after 6 months.

However a number of vascular tumours were found in treated males and females. The incidences appeared not to be dose-related, but this information is artificial due to the unknown numbers of early deaths.

Rat/oral carcinogenicity study

An oral study has also been conducted in the rat, combining a carcinogenicity and chronic toxicity study according to US EPA guideline 83-5. In the study (**Horner, 1996**) groups of 64 males and 64 female Alpk:AP_fSD rats were fed diets containing 0, 200, 600 or 2000 ppm PHMB for up to two years. Twelve rats of each sex from each group were designated for interim kills after 52 weeks, with the remaining animals continuing to terminal kills after 105 weeks. The mean dose received values for the 200, 600 and 2000 ppm groups respectively were 12, 36, 126 mg/kg/day for males and 15, 45, 162 mg/kg/day for females.

Administration of PHMB at a dose level of 2000 ppm produced treatment-related reductions in bodyweight, with the greater effects being seen in females (approximately 4-6% lower than controls throughout the majority of the study period in males and 10% lower than controls by week 91 in females with divergence from controls from week 35), and a slightly reduced survival in females during the second year of this study (0.92 Kaplan-Meier survival rate vs 1.0 in controls on week 52, 0.82 vs 0.90 on week 76 and 0.39 vs 0.52 on week 104). During the initial phase of the study, food consumption was reduced for both sexes at 2000 ppm, although slightly increased food consumption was recorded for females at this dose level during the second year of the study. It was, therefore, concluded that the high dose level of 2000 ppm was a satisfactory maximum tolerated dose level in this study.

Survival of females receiving 2000 ppm was slightly reduced from week 52. There was no treatment-related effect on survival (male rats), clinical signs, ophthalmoscopic findings or effects on any haematological or urinalysis parameters throughout the study. Slightly raised plasma alkaline phosphatase activity, predominantly in females receiving 2000 ppm, and a slightly

increased incidence of hepatocyte fat and spongiosis hepatis in males at this dose level, were possibly indicative of a mild effect in the liver (spongiosis hepatis in 7, 6, 7 and 14 males and in 3, 0, 1 and 2 females at 0, 200, 600 and 2000 ppm, respectively). A reduced incidence of peliosis hepatis was also observed in all treated male groups (peliosis hepatis in 11, 1, 3 and 3 males and in 2, 0, 0 and 2 females at 0, 200, 600 and 2000 ppm, respectively).

The original study summarised that there was no evidence of oncogenicity associated with administration of PHMB for 2 years at dose levels of up to 2000 ppm.

In response to the observation of a non-statistical increase in vascular tumour incidence in the liver at the top concentration (the trend test on haemangiosarcomas in female rats was positive only), a Pathology Working Group (with a comparable set up as mentioned for the mouse carcinogenicity study) review was commissioned to conduct an independent evaluation of the data (Busey, 1996). The PWG examined coded slides containing sections of liver from each of the male and female rats with a previous diagnosis of peliosis hepatis, haemangioma, or haeangiosarcoma.

The incidence of haemangioma and haemangiosarcoma in the liver based on judgement of the PWG is provided in the following table:

Table 19: Incidence of Haemangioma and Haemangiosarcoma in the Liver based on Judgement of the PWG

	Dose Group (ppm of PHMB)			
	0	200	600	2000
	Males			
Haemangiomas Original§	0/52 1/64	0/52 0/64	0/52 0/64	2/52 (4%) 1/64
Haemangio-sarcomas Original§	0/52 0/64	0/52 0/64	0/52 0/64	0/52 0/64
Combined	0/52	0/52	0/52	2/52 (4%)
	Females			
Haemangiomas# Original§	0/52* 0/64	0/52 0/64	0/52 0/64	2/52 (4%) 0/64
Haemangiosarcoma# Original§	0/52* 0/64*	0/52 1/64	0/52 0/64	1/52 (2%) 3/64
Combined	0/52	0/52	0/52	3/52 (6%)

No statistical analysis available in the PWG report (Busey 1996).

§ Original: Results from original study (Horner, 1996), taking 52 plus 12 animals killed after 52 weeks into account Fisher's Exact Test, trend test using Cochran-Armitage Test (Horner, 1996)

Statistical analysis of vascular tumour rates according to Peto's prevalence test was provided for female only in the USEPA analysis (USEPA, 2003) for hemangiomas and for haemangiosarcomas but not for combined tumours. Trend significance is denoted at control and pair-wise comparison with controls at the dose level. * $p<0.05$

No statistical analysis of these data is available in Busey (1996), but pairwise comparison and trend test was done in the original study of Horner (1996) (indicated as Original in Table 19). The PWG referred to historical control data at the study laboratory shows that only one haemangiosarcoma has been observed in the liver of control males out of 18 studies, no haemangioma in the liver of male

or female rats and no haemangiosarcoma in the liver of female rats have been observed in these studies. The incidence of vascular tumours in liver at high dose, although being low, was therefore above the historical control data in the present study.

Chemicals associated with the induction of haemangiomas and haemangiosarcomas in the liver of rats are uncommon. Notwithstanding this observation, haemangiosarcoma of the liver has been induced with several strongly carcinogenic chemicals including quinoline, tetrafluorethylene, vinyl fluoride, vinyl chloride, and vinyl bromide. In the studies that generated these data there was an associated increase in the incidence of non-neoplastic vascular lesions considered to be probable precursors of vascular neoplasms in the non-neoplastic regions of the liver. In contrast, in the study on PHMB there was no histologic evidence of an increased incidence of non-neoplastic vascular changes which would be indicative of a preneoplastic process. However, the absence of non-neoplastic vascular lesions in this study is not considered to be sufficient to negate the apparition of vascular neoplasms that are observed and their relation with the treatment.

The incidence of vascular neoplasms in the liver was much lower than that reported by the Study Pathologist at other sites such as mesenteric lymph node. The incidence of haemangioma and haemangiosarcoma at other sites based on judgement of Study Pathologist is provided in the following table 20:

Table 20: Incidence of Haemangioma and Haemangiosarcoma at sites other than liver

	Dose Group (ppm of PHMB)			
	0	200	600	2000
	Males			
Haemangiomas	11/52 (21%)	8/52 (15%)	5/52 (10%)	6/52 (12%)
In§: Ln mesenteric Spleen	 11 0	 7 1	 5 0	 6 0
Haemangio-sarcomas	1/52 (2%)	4/52 (8%)	1/52 (2%)	1/52 (2%)
In§: Kidney Ln cervical Ln mesenteric	 1 0 0	 1 0 3	 0 1 0	 1 0 0
Combined	12/52 (23%)	12/52 (23%)	6/52 (12%)	7/52 (13%)
	Females			
Haemangiomas	1/52 (2%)	1/52 (2%)	3/52 (6%)	3/52 (6%)
In§: Ln mesenteric	1	1	3	3
Haemangio-sarcomas	1/52 (2%)	0/52	0/52	0/52
In§: Ln mesenteric	1	0	0	0
Combined	2/52 (4%)	1/52 (2%)	3/52 (6%)	3/52 (6%)

No statistical analysis of vascular tumours at sites other than liver in the study report (Horner 1996).

Statistical analysis of vascular tumour rates according to Peto's prevalence test was provided for female only in the USEPA analysis (USEPA, 2003) for haemangiomas and for haemangiosarcomas but not for combined tumours. Trend significance is denoted at control and pair-wise comparison with controls at the dose level. * $p<0.05$

§ The incidences at other sites were reproduced by the rapporteurs from Busey (1996) who referred to the data published by the study pathologist of the study of Horner (1996).

The incidence of haemangioma and haemangiosarcoma at any sites (liver + other sites) based on judgement of PWG for liver and of Study Pathologist for other sites is provided in the following table:

Table 21: Incidence of Haemangioma and Haemangiosarcoma at any site

	Dose Group (ppm of PHMB)			
	0	200	600	2000
	Males			
Haemangiomas	11/52 (21%)	8/52 (15%)	5/52 (10%)	8/52 (13%)
Haemangio-sarcomas	1/52 (2%)	4/52 (8%)	1/52 (2%)	1/52 (2%)
Combined	12/52 (23%)	12/52(23%)	6/52 (12%)	9/52 (17%)
	Females			
Haemangiomas	1/52 (2%)	1/52 (2%)	3/52 (6%)	5/52 (10%)
Haemangio-sarcomas	1/52 (2%)	0/52	0/52	1/52 (2%)
Combined	2/52 (4%)**	1/52 (2%)	3/52 (6%)	6/52 (12%)*

Statistical analysis of vascular tumour rates according to Peto's prevalence test was provided for females only in the USEPA analysis (USEPA, 2003) and for combined hemangiomas and for haemangiosarcomas only. Trend significance is denoted at control and pair-wise comparison with controls at the dose level. * $p<0.05$, ** $p<0.01$

The incidence of females having vascular neoplasms at any site was statistically significantly increased at high dose. This increase was mainly due to higher incidence of haemangiomas at high dose level (3 in the liver and 3 at other sites). Historical control data shows that the range of hemangiosarcomas at all sites in rats was 0-1.9% for males (mean 0.7%) and females (mean 0.4%)**.** No data is available for combined vascular tumours.

The opinion of the PWG was that overall weight of evidence indicated that the slightly higher number of group 4 male and female rats having vascular neoplasms of the liver is not associated with the dietary administration of PHMB.

With respect to vascular tumours in rats, RAC concluded that evidence from rat carcinogenicity study is not sufficient to conclude a clear treatment-related effect.

There was another rat oral carcinogenicity study (**Berry et al., 1977**). Due to infections and less than 50% of animals were alive at the end of study dossier submitter regarded this study inacceptable for the CLH proposal and Biocide CAR. In this study groups of 60 male and 60 female rats (strain not specified) were fed Baquacil SB at levels of 0. 200, 1000 and 2000 ppm PHMB. The

study was terminated at 124 weeks when 80% mortality had occurred. Interim kills were undertaken at 52 and 104 weeks to provide tissue pathology data at these points.

Two outbreaks of respiratory infection occurred during the study, one at 70 weeks and the other at 103 weeks. The symptoms of both infections were snuffling, rapid and laboured respiration and croaking and were more severe at 103 weeks. The incidence of numbers of animals affected was evenly distributed between groups.

The incidence of mortalities was similar across all groups throughout the study. The respiratory infection at week 70 and week 103 caused a rise in the mortality rates.

No observable clinical effects were produced by the compound and apart from a slight anaemia in the top dose at 104 weeks, no adverse changes were seen. The administration of Baquacil SB caused some growth depression in treated animals. Food consumption remained lower in those animals given 200 or 1000 ppm PHMB than in controls throughout the study and the slight reduction in bodyweight, compared to controls, in these animals was ascribed therefore to a palatability effect. At 2000 ppm PHMB, the effect on bodyweight appeared to be due to the toxicity of the compounds. (RAC rapporteur's view is that it seems unplausible to explain lower consumption in the lower doses due to bad palatability, and at high dose due to toxicity. Palatability should be expected to accelerate with concentration in diet.)

Long-term exposure to PHMB was not related to toxic or carcinogenic effects. Haemangiomas were found at interim kills at week 52 in 1/12 male rats (mesenteric lymph nodes) at 200 ppm and 1/12 male rats at 200 ppm (cervical lymphnodes), at interim kill at 104 weeks in 2/12 males at 1000 ppm (mesenteric lymphnodes) and in 1/8 females at 200 ppm (uterus) as well as at the end of study at week 124 1/20 males at 1000 ppm (mesenteric lymph nodes), 1/19 males at 2000 ppm (spleen) and one haemangiosarcoma at week 104 in 1/21 males at 2000 ppm (mesenteric lymph nodes). No vascular tumours were seen in controls.

In this document also a dose range finding in rats was mentioned (Clapp, 1973, no report available). Rats were fed diets containing PHMB at a range of concentrations from 20 to 10000 ppm. The 10000 ppm level caused weight loss and markedly reduced food consumption. The 2000 ppm level caused less marked effects and it was decided to use this concentration as the top dose for the main study. (No further details available).

5.7.2 Carcinogenicity: inhalation

No data available

5.7.3 Carcinogenicity: dermal

An 80-week skin painting study was conducted using a 20% aqueous formulation of PHMB (**Clapp, 1977b**). The study was conducted pre-GLP and prior to the development of any published guidelines. Dosing formulations (ethanol/water) of PHMB were applied to the shorn backs of groups of 50 male and 50 female Alpk:AP$_f$CD-1 mice at dose levels of 0 (control), 0.6, 6.0 or 30 mg PHMB/mouse/day (corresponding to a PHMB concentration in the vehicle of 0, 0.2%, 2.0% and 10.0% and approximately equivalent to 0, 15, 150 or 750 mg PHMB/kg/day), 5 days per week for 80 weeks. Clinical observations (including ophthalmoscopy), bodyweights and food consumption were recorded. All animals were subjected to a *post mortem* examination. A full range of tissues was taken for histopathological examination.

It was clear that the dose level of 30 mg PHMB/mouse/day exceeded the Maximum Tolerated Dose (MTD) based on excessive mortality (76-78% of animals dying prior to study termination) and

reduced bodyweight gain in both sexes (up to 50% reduction). Furthermore, noticeable irritation was seen immediately following application. This high incidence of irritation was exaggerated during week 76 when the undiluted PHMB solution was applied to the skin by error. For males given 6.0 mg PHMB/mouse/day there was only a transitory skin irritant effect at week 25, and also a reduction in bodyweight from week 18 (up to 7%) which disappeared over the second year of the study.

There was no evidence of a carcinogenic effect of PHMB at dose levels up to 6.0 mg/mouse/day. The higher dose of 30 mg/mouse/day greatly exceeded the MTD. At this dose there was a slight increase in liver tumours consisting of hepatocellular adenoma in 4 animals versus 1 in the controls, haemangioendothelioma in 3 animals versus 1 in the controls, and angiosarcoma in 3 animals versus 1 in the controls. Incidence of vascular tumours in the liver are summarised in the table below.

Table 22: Incidence of Animals with vascular tumours in the liver

	Dose Group (mg/kg/d PHMB)			
	0	15	150	750
	Males			
Haemangiomas	0/50	0/49	0/50	2/50 (4%)
Haemangio-sarcomas	1/50 (2%)	0/49	1/50 (2%)	1/50 (2%)
Combined	1/50 (2%)	0/49	1/50 (2%)	3/50 (6%)
	Females			
Haemangiomas	0/49	0/50	0/50	1/49 (2%)
Haemangio-sarcomas	0/49**	0/50	0/50	2/49 (4%)*
Combined	0/49	0/50	0/50	3/49 (6%)

No statistical analysis of vascular tumours in the liver reported in the study report (Clapp 1977b).

Statistical analysis of vascular tumour rates in the liver according to Peto's prevalence test was provided for female only in the USEPA analysis (USEPA, 2003) for hemangiomas and for haemangiosarcomas but not for combined tumours. Trend significance is denoted at control and pair-wise comparison with controls at the dose level. * $p<0.05$, ** $p<0.01$

The incidence of females having vascular neoplasms at any site was statistically significantly for trend (USEPA 2003). No compound-related histopathological changes were seen at 0.6 or 6.0 mg PHMB/mouse/day.

It should be noted that a number of the animals in the high dose group were found to have hepatitis. This was assumed to be related to infection with *Helicobacter hepaticus*. In general Helicobacter infection might be associated with an increased incidence of hepatitis and hepatocellular neoplasms. However, adenomas were present in all groups with only a slight increase in both males and females at the highest dose and there was no increase in hepatocellular carcinoma. Therefore, the potential confounding effect of the hepatitis is thought to be of equivocal relevance.

5.7.4 Carcinogenicity: human data

No data available.

5.7.5 Other relevant information

A mechanistic study (**Kamendulis, 2008**) was performed to investigate a possible mechanism of induction of liver haemangiosarcomas in mice.

In vivo, male C57Bl mice (5/group) were given diets containing 0, 100, 200, 400, 1200, or 4000 ppm PHMB for 7, 14, or 28 days. Immunohistochemical detection of bromodeoxyuridine (BrdU) in mouse liver was used to quantify cell proliferation in liver endothelial cells. Liver hepatoxicity was assessed by measuring alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma obtained at sacrifice. Plasma endotoxin levels were quantified using an endotoxin assay kit. Oxidative stress was measured by detection of 8-Hydroxydeoxyguanosine (OH8dG) in isolated DNA from livers.
PHMB did not induce hepatotoxicity at any concentration or time point. At 4000 ppm PHMB decreased transiently bodyweight and induced thinning of the stomach wall but at 28 days of exposure, no effect on body weight or liver weight was observed at any dose. PHMB increased cell proliferation in a dose-responsive manner at 1200 and 4000 ppm. Cell proliferation was also increased at 1200 ppm PHMB following 14 days exposure. PHMB increased plasma endotoxin, a known activator of macrophages, at 1200 and 4000 ppm for 28 days and at 100 and 200 ppm for14 days but not for longer duration.

In vitro, to examine how the interaction between macrophages and liver endothelial cells can induce endothelial cell proliferation, RAW 264.7 mouse macrophages were co-cultured with SVEC-10 mouse liver endothelial cells in various experimental conditions: 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 TNFα and IL-6 in cell culture medium as quantified by ELISA.
In vitro, the study showed that PHMB had no direct effect on liver endothelial cell proliferation, PHMB did not activate macrophages and presence of PHMB did not potentiate cell proliferation induced by LPS-activated macrophages.

Collectively, these results suggested that the effect of PHMB seen *in vivo* on liver endothelial cell growth at 1200 and 4000 ppm for 28 days was not produced through a direct effect, but rather through an indirect effect. Release of endotoxins at the same doses suggested that endotoxin-mediated activation of macrophages may be involved. However, the causal relationship of these two events was not demonstrated and presence of endotoxins at intermediate duration at lower doses questioned its relevance. Besides, other mechanisms of action can not be excluded and were not investigated. In this study, the increase in endothelial cell growth, as measured by DNA synthesis, occurred in a dose-responsive manner with a threshold at 400 ppm.

5.7.6 Summary and discussion of carcinogenicity

Main results are summarised in Table 23.

Table 23: Summary of relevant chronic toxicity/carcinogenicity

Route	**Dura-tion**	**Species Strain**	**Dose levels** [mg/kg bw/day]	**Results**	**LO(A)EL**	**NO(A)EL**	**Reference**

					[mg/kg bw/day]		
Oral (dietary)	104 weeks	Rat: (Alpk: AP_fSD) 64 males and females per group	0, 200, 600 or 2000 ppm (0, 12, 36 or 126mg/kg males: 0, 15, 45 or 162 mg/kg females); daily in the diet	Three vascular neoplasms (two haemangiomas and one haemangiosarcoma) were observed in the livers of the females at 2000 ppm, and two haemangiomas in the males at 2000 ppm.	126 –– 162-	36 – 45 (for carcinogenic effects)	Horner, 1996
Oral (dietary)	104 weeks	Mouse: (C57BL/10JfCD-1/Alpk) 55 male and 55 female per group	0, 400, 1200 0r 2000 ppm (55, 167 or 715 mg kg males: 69, 217 or 856 mg/kg females); daily in the diet	At 4000 ppm bodyweights were up to 20% (M) and 15% (F) lower than controls; bodyweight gain was reduced by 35-42% (M) and 22-33% (F); mortality increased in female. Therefore, 4000 ppm was considered clearly in excess of an MTD. The main treatment related observation in males and females at 4000 ppm was anal swelling. There was an altered tumour profile at 4000 ppm, with decreases in lymphosarcomas (M&F) and pituitary gland adenomas (F). Increases were seen in squamous cell carcinomas of the recto-anal junction (M&F) and gall bladder papillomas (M). Vascular tumours, mainly haemangiosarcomas, were also increased. The anal swelling (and also discharge and sores) observed at 4000ppm correlates with the findings of inflammation, squamous epithelial hyperplasia and squamous metaplasia in the recto-anal region. PHMB is poorly absorbed from the diet, and concentrates in the recto-anal region before evacuation. Consequently, the effects seen are attributable to chronic irritation caused by high concentrations of an irritant compound. This irritant effect (through biliary excretion) is also the likely cause of the two gall-bladder papillomas seen in males. In mice receiving 1200 ppm there was a variety of non-neoplastic changes in the recto-anal junction of an inflammatory nature. In addition mice receiving 1200 ppm showed non-neoplastic changes in the liver (both sexes) and gall bladder (females only) and male mice showed an increase in liver haemangiosarcomas.	167 for male and 217 for female	55 for male and 69for female	Milburn, 1996

Dermal	80 weeks	Mice (Alpk: AP_fCD-1) 50 males and 50 females per group	0, 0.2%, 2.0% or 10.0% (equivalent to 0, 0.6, 6.0 or 30 mg per mouse per day and to 0, 15, 150 or 750 mg/kg/d); 5 days per week for 80 weeks	The highest dose (30 mg PHMB) produced irritant effects on the skin and led to a generally poor condition in the mice which was reflected by bodyweight loss and a high mortality incidence. It was clear that this does exceeded the MTD based on excessive mortality. There was only a transitory skin irritant effect on male mice receiving 6.0 mg per day and some reduction in bodyweight gain. There was no effect at 0.6 mg per day. PHMB did not give rise to carcinogenic effects on the skin but a statistically significant increase of liver haemangiosarcomas is observed in females at the high dose. No abnormal histopathological changes were noted at 0.6 or 6.0 mg per mouse per day.	Local and systemic non-carcinogenic effects: 150 mg/kg/d Carcinogenic effect: 750 mg/kg/d	Local and systemic non-carcinogenic effects: 15 mg/kg/d Carcinogenic effect: 150 mg/kg/d	Clapp, 1977b

Local carcinogenic effects

PHMB induces squamous cell carcinomas in the recto-anal junction in mice at the highest dose that is reported to exceed the MTD. The induction of these tumours is considered related to chronic inflammation due to the substance irritative properties that induced inflammation at all doses and squamous hyper/metaplasia at the mid and high dose. Considering the combination of arguments that these tumours are due to a secondary mode of action with the implication of a practical threshold such as chronic stimulation of cell proliferation and that they are observed only at a high dose exceeding MTD, these tumours were not considered relevant for classification by dossier submitters.

Local contact to PHMB by biliary excretion can be assumed for two gall bladder papillomas observed in male mice in this study. Due to the lack of other supportive data, dossier submitter considered the evidence of PHMB-related carcinogen effect is very limited.

In contrast, RAC considers the MTD as of equivocal relevance for tumours that were induced at the site of contact. The fact that chronic inflammation and squamous cell hyperplasia were already observed in low, respectively in mid dose groups and increased in a dose-related manner support the evidence that squamous cell carcinomas at the recto-anal junction could be attributed to chronic inflammation and subsequent hyper/metaplasia that precedes tumour development specific for PHMB. The observed squamous cell carcinomas are considered as indicative of a potential for local tumourgenicity.

Gall bladder papillomas in two high dose mice were also interpreted as being related to a local chronic inflammatory response following biliary PHMB excretion. Epithelial hyperplasia is interpreted as related precursor lesion that were only seen in treated groups (all male dose groups, females at mid and high dose) at dose-related incidences. Since PHMB has irritative properties to any surface epithelium as shown in the eye, at the skin and the upper respiratory tract, a relevance for humans could not completely be excluded. These tumours at the site of contact are PHMB-

related and due to the precursor lesions (chronic inflammation) and squamous hyperplasia and metaplasia they are likely to be caused by a thresholded mode of action.

Classification criteria say that a careful evaluation for human relevance is needed for tumours occurring only at site of contact and/or only at excessive doses. A questionable relevance may be given if there is lack of corresponding tissue in humans (which is not the case here) due to the high dose direct effect on the tissue, any occurrence of other tumours at distant sites must also be considered.

Criteria consider persistent irritation/inflammation, tissue erosion and regenerative hyperplasia and tumour development e.g. following urinary bladder stone formation. Such lesions are not relevant for humans and thus are not relevant for classification, if mode of actions (for urinary bladder: crystal formation) are identified that do not operate in humans. It is recommended that the existence of a secondary mechanism of action with the implication of a practical threshold (e.g. due to chronic stimulation of cell proliferation) may lead to a downgrading of a Cat 1 to a Cat 2 classification.

Regarding local tumour responses of PHMB treatment in the gallbladder and at the recto-anal junction, chronic inflammation and regenerative hyperplasia is likely to be the thresholded mode of carcinogen action. As a default assumption the mode of action can be assumed to operate in humans as well.

Overall local tumour response gives supportive evidence of PHMB carcinogenic potential.

Systemic tumour response

Induction of vascular tumours, mainly in the liver, is reported in three valid carcinogenicity studies performed with PHMB :

- In the mice dermal study (Clapp, 1977b), a statistically significant increase in the incidence of liver haemangiosarcomas is observed in females at the high dose of approximately 750 mg/kg PHMB. This dose is considered to exceed the MTD. Although it is remarkable that the same tumour type occurred as in other studies.
- In the mouse oral study (Milburn, 1996), a statistically significant increase in the incidence of haemangiosarcomas at any site was observed in males and females at the high dose of 4000 ppm (715 and 856 mg/kg PHMB respectively), with incidence of haemangiosarcomas above internal control groups and above laboratory historical control data.

  Significant increases at any site can mainly be contributed to tumours in the liver. In the liver, there was a clear increase in vascular tumours in males and females at the high dose. Although statistical analysis is not available for the liver tumours, incidences of haemangiomas, haemangiosarcomas and the incidences in combined haemangioma/haemangiosarcomes were elevated at the 4000 ppm dose level. Increased rates of benign and malignant tumours of this type strengthen the evidence for a PHMB-related carcinogenic potential. The fact that haemangiosarcomas occurred at higher rates than haemangiomas supports the malignant character of the substance in this study. A moderate increase of liver haemangiosarcomas was also observed at mid-dose (1200 ppm – 167 mg/kg PHMB) in males. Although statistical analysis is unknown and historical control data are not available for this value, this increase is considered biologically significant compared to controls and can be attributed to treatment.

RAC considered the proposal of Industry and dossier submitter that interpretation of vascular tumours at the high dose (4000 ppm) should be done in the light of exceeding the MTD. Facts to support that MTD was exceeded are increased mortalities and decreases in body weight (gain) in males and females at 4000 ppm, these effects were interpreted as indicative of systemic toxicity.

Supportive evidence could be derived from the 1 year toxicity study in dogs (Horner, 1995) where 3 of 4 dogs showed severe signs of toxicity (not specified) after receiving 4500 ppm PHMB with diet and dosage was reduced to 3000 ppm from week 11 onwards. PHMB induced in dogs at high dose cell damage in the liver (eosinophilic cytoplasmatic inclusion bodies, single cell necrosis, liver cell swelling) and in kidney and testis. From this chronic study in the dog it appears that 4500 ppm was clearly above MTD. However this species might be more sensitive than mice and rats. A dose range 28 day study in rats (Clapp, 1973) allowed dosing up to 10000 ppm PHMB in diet, which caused lower body weight and reduced food consumption.

RAC acknowledged that the high dose of 4000 ppm PHMB caused reduction in body weight gain in mice. Bodyweights were significant lower throughout the study and up to 20% (males) and 15% (females) lower than those of concurrent controls in the second year of the study. In terms of bodyweight gain, there was a significant reduction throughout the study and reached 35-42% (males) and 22-33% (females) compared to the controls during weeks 53-79. Partly this could be contributed to a higher dose per kg bodyweight/d during the first 13 weeks (800-900 mg/kg bw/d in males and 900-1000 mg/kg in females) than later on. Reported as an unusual effect, food consumption was increased throughout the study in males and females at 4000 ppm. Food utilisation was significantly less efficient compared to controls (most marked at the start of the study, week 1 to 4: -64% in males, -47% in females). It is noteworthy that no other clinical signs of treatment-related toxicity was observed throughout the study.

Industry discussed that mortality rates at high dose groups were due to high systemic toxicity while the study report correlated the occurrence of haemangiosarcomas as the most frequent factor to deaths. However, for high dose males, showing the strongest increase in vascular tumours, the overall mortality rate at the end of study was similar to controls. Only during the period of week 30 to week 90 the mortality rate was increased above controls. For a high number of animals that intercurrently died, mortality was identified due to liver haemangiosarcomas. Therefore it is uncertain how many mortalities (of animals not bearing liver haemangiosarcomas as cause of death) could be contributed to systemic toxicity.

Mortalities in high dose females increased from week 26 onwards. Again, haemangiosarcomas were often identified as cause of death. If haemangiosarcoma-bearing animals at high dose were distracted from the overall number of intercurrent deaths (Table 17) the mortality rates at high dose are similar or even lower than in the control groups. Taking into account the absence of any other clinical sign of toxicity this raises uncertainty about whether mortalities in male and female mice at high dose were indicative of general toxicity.

Comparison of data on PHMB with other biguanides reveals some indication that members of the biguanide class decrease serum glucose level (http://en.wikipedia.org/wiki/Biguanide). 1,1-dimethylbiguanide (Metformin) which is used for antihyperglycemic therapy of diabetes

mellitus type 2 and treatment of overweight patients, has been shown to cause decreased intestinal glucose absorption and to suppress gluconeogenesis and ATP production in hepatocytes (Musi et al, 2002, Foretz et al., 2010). Metformin exerts its effects by activation of AMP-activated protein kinase (AMPK), which is a major regulator of cellular and whole-body energy homeostasis and leads to the inactivation of acetyl CoA carboxylase. Stimulation of AMPK increases glucose uptake in muscle while also inhibiting hepatic glucose production, cholesterol and triglyceride synthesis, and lipogenesis.

This observation supports assumption that the reduced body weight gain at high doses of PHMB like other biguanide members may be mediated by the hypoglycaemic effects that is characteristic for this substance group.

In conclusion on this issue, MTD could be viewed of being reached applying reduced growth as the only indicator. However, data on mortalities and tumour-related cause of deaths at high dose and the absence of any clinical sign of toxicity do not support a link of mortalities to treatment-related nonspecific toxicity. Knowledge from other biguanides that have hypoglycaemic activities might also explain the low body weight gains. Whether or not MTD was exceeded at high dose level remains uncertain.

It should be mentioned that guidance on CLP regulation (Chapter 3.6.2.3.2 (j)) recommends that 'if a test compound is only found to be carcinogenic at the highest dose used in a lifetime bioassay, this could be an indication of a confounding effect of excessive toxicity. This may support a classification of the test compound in Category 2 or no classification'.

Nevertheless a dose-related increase in vascular tumors in the liver at the mid dose which is below the (suggested) MTD was also seen. These tumours should also be regarded as being related to the PHMB treatment.

Incidences of haemangiosarcomas in control groups are within the ranges reported for internal laboratory controls. As there is no reason to assume invalidity of control data, tumour incidences at the mid dose level are higher than those in control groups for male and female mice. Although no statistical analysis on liver tumours was available, the increased incidences at mid dose groups are in line with the view that vascular tumours in the liver at mid and high dose level are dose-related and supportive for the conclusion that tumours were related to PHMB treatment.

RAC considered it unlikely to explain higher rates of vascular tumours in the liver at 1200 ppm PHMB and above by chance and concluded that data from the mouse carcinogenicity study of Milburn (1996) give some evidence of carcinogenic potential.

- Industry asked for consideration of the oral mice study of Clapp et al (1977a) that was not considered as reliable in the original CLH proposal by dossier submitter and has been added by rapporteurs for transparency. It should be noted that this study had a number of flaws. Mainly fighting among male animals was related to high mortality during the first 6 months of the study. However, the numbers of affected animals was not given. Although the study report concluded that no treatment-related increases in tumours were observed, there were a number of vascular tumours that were exclusively observed in treated animals. In the liver one haemangiosarcoma was observed in one male and one female at 1000 ppm PHMB and one haemangioma in one male and one female at the 200 ppm PHMB. Overall, tumour data

are not very reliable due fighting-related mortalities. However, the study can not be regarded as a 'negative' study outweighing the positive findings from the Milburn study.

- In the rat oral carcinogenicity study (Horner, 1996), a low incidence of haemangiomas (4%) occurred in males and females receiving 2000 ppm (162 mg/kg PHMB) while no vascular tumours were seen in control groups. A single haemangiosarcoma of the liver was seen in high dose female (PWG analysis, Table 19). Due to the absence of liver tumours in female controls, vascular tumors in treated females showed a significant trend, however pairwise comparisons did not reveal significant differences among test groups. There was no evidence of dose-related and/or time-related increases in non-neoplastic vascular lesions (such as endothelial cell proliferation) in this study except a higher incidence in spongiosis hepatis at 2000 ppm (males only). No other data are available that give reliable information on non-neoplastic liver lesions in rats since the short-term inhalation studies are not informative with respect to liver lesions and no oral short-term studies are available for rats (and mice). This kind of tumours is rare in control rats. The incidence of vascular tumours in the liver at the high dose, albeit it was low, exceeds the historical controls in both males and females.

  With origin from endothelial cells, vascular tumours are classified as 'systemic' tumours, which are known to occur in a range of organs and are mainly found in liver, spleen, bone marrow and lymph nodes. In rats of the Horner study, haemangiomas and haemangiosarcomas were also observed at other sites than the liver. In this study haemangiomas were frequently seen in mesenteric lymph nodes, where incidences in males of the control groups were clearly above those in dose groups (21% (control) compared to 15%, 10%, 12% in dose groups, Table 20). Haemangiomas in female rats were elevated in mid and high dose groups (each with 3 tumours/52 females = 6% compared to 2% in controls), but no haemangiosarcomas were seen in dose groups and one in the controls.

  Diverging curves on incidences were seen when vascular tumours at all sites were considered. Without any dose-relationship incidences in control males were high (21%) compared to lower rates in dose groups (15%, 10%, 13%). Opposing to this, incidences of haemangiomas at all sites appeared to increase with dose in females (2% (control), 2%, 6%, 12% in dose groups, Table 21, based on PWG results). Incidences of haemangiosarcomas at all sites were the same in control and high dose groups for both sexes.

  With respect to vascular tumours in rats, the RAC concluded that evidence from rat carcinogenicity study is not sufficient to conclude a clear treatment-related effect due to the facts that overall increases in vascular tumours of the liver was small and high incidences of spontaneous haemangiomas in lymph nodes weaken the strength of evidence that vascular tumours at the high dose are treatment-related.

- A post-operational statistical analysis by Sielken and coworkers dated 19 October 2010, was made available to RAC in November 2010. RAC considered the document and concluded that it does not have an impact on RAC's assessment.

- Mode of action

  A mechanistic study in mice (Kamendulis, 2008) investigated a hypothetical mechanism of action and suggested that liver haemangiosarcomas are induced by an indirect mechanism involving release of endotoxins from gastrointestinal tract into liver and bloodstream

subsequently to action of PHMB on gram-negative bacteria. Endotoxin may activate Kupffer cells potentially resulting in endothelial cell proliferation and ultimately leading to neoplasia. However, the causal relationship between endotoxin release, Kupffer cell activation, endothelial cell proliferation and tumour induction is not demonstrated and the presence of endotoxins at doses below doses inducing cell proliferation questioned its relevance.
Despite the inclusive result on the effect of endotoxins, PHMB induced cell proliferation in liver endothelial cells; the putative cell of tumour origin for haemangioma/angiosarcoma; in mice receiving 1200 ppm following 14 or 28 days of treatment or receiving 4000 ppm following 28 days of treatment (no data available for day 14).

Besides, other mechanisms of action were not investigated and cannot be excluded. It is noted that PHMB is not considered genotoxic and the mechanistic study establishes a NOEL for liver endothelial cell proliferation at 400 ppm after 28 days of dietary exposure in mice, which is consistent with the NOEL for tumour induction in the oral mouse carcinogenicity study.

PHMB increases the incidence of benign and malign vascular tumours in male and female mice by oral - and taking the lower strength of evidence due to MTD dosing into account - also by dermal route. The tumours are induced mainly in the liver, which is one of the target organs of PHMB and the increase is clearly seen at the high oral dose of 4000 ppm PHMB, which was reported to be above the MTD. However interpretation whether MTD was exceeded has uncertainties since the MTD was questioned in the light of high tumour-related mortalities and the assumption that reduced body weight gain could eventually be contributed to a hypoglemic effect of PHMB. Dose-related increased incidences of vascular tumours were also observed at doses below the proposed MTD (mouse oral study at mid-dose). These increases are not interpreted to be incidental with regard to the dose-response relationship of vascular tumours at mid and high doses, the lower incidence or even absence in control groups, and some evidence for consistency across administration routes. They are considered biologically significant and attributed to treatment.

Additional concern given from squamous cell carcinomas in the recto-anal region and from papillomas in the gallbladder of mice, which are attributed to the chronic inflammation and regenerative hyperplasia might indicate that PHMB may exert (local) tumourgenicity at sites of contact at concentrations inducing excessive inflammatory toxicity.

Relevance for humans could not be excluded and evidence of (local) carcinogenicity is interpreted to give supportive evidence of PHMB carcinogenic potential.

RAC is aware that the overall evidence on carcinogenic potential of PHMB is not strong. The criteria say about Category 2 ‘it is recommended if there is limited evidence of carcinogenicity. Data suggest a carcinogenic effect but are limited for making a definitive evaluation because e.g. a) the evidence of carcinogenicity is restricted to a single experiment b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential or d) the evidence is restricted to studies that demonstrates only promoting activity in narrow range of tissues or organs.’

With respect to PHMB the evidence of carcinogenicity (systemic and local) is mainly from a single experiment (mouse oral carcinogenicity study), but there is supporting evidence from other studies in mice (criteria (a) is valid). There are remaining uncertainties about interpretation with respect to the MTD (criteria (b) is valid).

PHMB is not genotoxic *in vitro* and *in vivo*, but taking into account that the overall evidence on carcinogenicity is mainly on the evidence from one study in one species and no mode of action has been identified a classification as carcinogenic category 3; R40 (CLP Carc 2 – H351) is warranted.

In absence of carcinogenicity data by inhalation, it is proposed to allocate the general hazard statement H351 without indication of the route of exposure.

In the weight of evidence, as a clear treatment-related increase in vascular tumours is induced in one species only and considering the lack of mutagenicity, justification is given that classification Carc 1B is not appropriate for PHMB.

5.8 Toxicity for reproduction

5.8.1 Effects on fertility

A summary of the main results on effect on fertility of PHMB is provided in Table 24.

Table 24: Summary of Reproductive Toxicity

Route of exposure	**Test type Method Guideline**	**Species Strain Sex no/group**	**Doses Test material (mg/kg)**	**Critical effects**	**Reference**
Oral in the diet	Two-generation study Consistent with guideline procedures for a multi-generation reproduction study.	Rat; Male and Female 26/sex	200, 600 and 2000 ppm Equiv. to 23-24, 70-71 and 239-249 mg/kg/d bw in males Equiv. to 25-26, 77-79 and 258-270 mg/kg/d bw in females	Lower bodyweights at the top dose in the F_0 and F_1 animals during the premating period. No effect on reproductive parameters or on offspring growth and development. Decreased relative epididymis weight (-4% and -8%) was observed in the F0 male rats at 600 and 2000 ppm concentration. Decreased absolute epididymis weight was observed in the F2a pups at 200 and 600 ppm (-32 and -40%) but no effect was seen on relative weight. The statistically significant increase in relative testes weights in F1 males receiving 600 ppm was considered incidental in the absence of any similar findings in rats receiving 2000 ppm. Absolute testes weight in the F2a pups was also decreased by 23% at 600 ppm but without effect on relative weight and effect at the highest dose. Besides, these findings were considered to be without toxicological significance as no histopathological changes were observed in these tissues. There was no apparent detrimental effect of PHMB treatment on pup survival. However the number of litters with all pups surviving to day 22, as a percentage of all litters, was lower for both F1a and F2a offspring. The number of pup deaths from days 1-5 post-partum was high in relation to pup deaths from days 5-22. However this was attributed to maternal stress, possibly compromising early nursing of the pups due to environmental disturbances (building work in an	Milburn, 1995

				adjacent animal block). Since satisfactory numbers survived to weaning the early increased death percentage was not considered to be treatment related.	
	Three-generation study	Rat; 10 males and 20 females /group	0, 200, 650 and 1300 ppm Approx. 0, 20, 65 and 130 mg/kg bw/d	No effects attributable to the administration of 20% PHMB were observed in the evaluation of parental food consumption values, survival rates, clinical findings, pregnancy rates, or reproduction data. There were no meaningful differences between body weight data of the control and test parental animals except for slightly decreased body weight gains in the P_3 mid- and high-level males when compared to that of the P_3 control males ; this finding is suspected to be associated with compound administration. In addition, evaluations of the various reproductive indices, sex ratios, and body weight data of the foetuses taken by ceasarean section and the offspring maintained through weaning revealed no meaningful differences between the control and treated groups. Necropsy of weanlings did not reveal any compound-related gross pathology. No findings indicative of embryotoxicity or teratogenicity were noted in the foetuses taken by ceasarean section.	Trutter, 1977

5.8.2 Developmental toxicity

A summary of the main results on developmental toxicity of PHMB is provided in Table 25.

Table 25: Summary of teratogenicity

Route	**Test type Method Guideline**	**Species Strain Sex no/group**	**Exposure Period**	**Doses** [mg/kg bw/day]	**Critical effects**	**Reference**
Oral (dietary)	Consistent with OECD 414	Rat; Females; 20-22	Throughout the gestation period (0-20 days)	13, 54, or 112	Dams – weight gain significantly reduced at 54 and 112 mg/kg/day (-23% of controls at both dose). Food consumption also significantly reduced in these groups. Foetuses – No dose-related effects were observed on pre-implantation and post-implantation loss and on foetal and litter weights. Increase in extra ribs at 112 mg/kg which is indicative of foetal toxicity caused by maternal toxicity and not a teratogenic event.	**Hodge, 1976**
Oral	Consistent with OECD 414	Rabbit; New Zealand White; Females; 20	Days 8 through 20 of gestation.	10, 20, and 40	Dams – Increased mortality at the top dose (6 dams). Reduced food intake (-23% compared to controls between GD8-14, $p<0.01$) and reduced bodyweight gain at the top dose. In animals which died on study there were macroscopic changes in the stomach or caecum consistent with irritation and inflammation at site of contact. Signs of recovery were evident post-dosing when this group showed	**Brammer, 1993**

Route	Test type Method Guideline	Species Strain Sex no/group	Exposure Period	Doses [mg/kg bw/day]	Critical effects	Reference
					increased food consumption and final body weights similar to controls. Foetuses – There was no effect of PHMB on the number, growth or survival of the foetuses *in utero* except a slight increase in pre-implantation loss observed at 40 mg/kg (21.8±25.6 vs 13.1±15.2 in controls) and a significant increase in post-implantation loss observed at 20 mg/kg (11.4±19.7% vs 6.1±8.4% in controls) attributed to an increase in early intra-uterine deaths. But the difference in pre-implantation loss at 40 mg/kg was not statistically significant and could not be related to PHMB as the dosing period began after implantation. The post-implantation loss at 20 mg/kg was not seen at the highest dose and in the absence of dose-response relationship, this effect was not attributed to treatment. There was no evidence for teratogenicity. The percentage of foetuses with unossified 5th sternabrae or with fused 4th and 5th sternabrae was increased at the top dose, but this effect is considered not related to treatment.	

5.8.3 Summary and discussion of reproductive toxicity

In the 1-year repeated toxicity study, testicular tubular degeneration was noted in 2/4 dogs at the highest dose (169 mg/kg reduced to 108 mg/kg). The initial dose also induced severe signs of toxicity that justified the sacrifice of 3/4 males at week 9, 15 and 15, respectively and provoked reduction of dosing on week 11 or 12. Besides, no effects on reproductive parameters were observed in a rat two-generation up to approximately 250 mg/kg and in a rat three-generation study up to 130 mg/kg. Some effects on the weight male reproductive organs were identified in the two-generation study but in the absence of histological effects and in some case dose-response, these effects were not considered of toxicological significance and no classification is proposed for fertility.

No evidence of foetotoxicity and teratogenicity is observed in prenatal studies in the rat and the rabbit and no classification is proposed for developmental toxicity.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

The test substance was 99% PHMB in all tests mentioned below.

6.1 Explosivity

The available tests (Schofield, 2007) were performed in accordance with EEC- directive A 14 "Explosive properties" and were conducted in compliance with the UK Principles of Good Laboratory Practice. The test substance was submitted to the effect of heat under confinement (thermal sensitivity),to impact and friction (mechanical sensitivity).

Thermal Sensitivity:

No explosions were observed using the 2 mm orifice plates, i.e Limiting Diameter < 2mm. The test substance is not classified as explosive in terms of its thermal sensitivity.

Mechanical Sensitivity (shock):

Limited Impact Energy > 40 Joules. The test substance is not classified as explosive in terms of its mechanical sensitivity with respect to shock.

Mechanical Sensitivity (friction):

Limited Load > 360 Newtons. The test substance is not classified as explosive in terms of its mechanical sensitivity with respect to friction.

No classification for explosivity is proposed for PHMB.

6.2 Flammability

The available tests (Schofield, 2007) were performed in accordance with EEC-directive A10" Flammability (solids), A12 "Flammability (contact with water)", A13 "Flammability (pyrophoric properties) and A16 "Auto-inflammability- Relative Self Ignition Temperature for solids" and were conducted in compliance with the UK Principles of Good Laboratory Practice.

Flammability (solids) A10:

The test did not propagate the combustion. So PHMB is not classified as highly flammable.

Flammability (contact with water) A12:

The test does not evolve highly flammable gases on contact with water. So PHMB is not classified as highly flammable in terms of its reactivity with water.

Flammability (pyrophoric properties) A13:

The test substance does not spontaneously ignite on contact with air at ambient temperature. So PHMB is not classified as highly flammable in terms of its pyrophoric properties.

Auto-inflammability- Relative Self Ignition Temperature for solids A 16:

The test substance did not ignite below 400°C, the upper limit of the test.

PHMB was found to be not flammable and it has no self-ignition temperature.

No classification for flammability is proposed for PHMB.

6.3 Oxidising potential

According to the EEC-directive A17 “Oxidising properties”, an analysis of the chemical structure of PHMB shows that it does not possess oxidizing properties and will not give rise to highly exothermic reactions when in contact with others substances, particularly flammable ones, in the manner in which recognized oxidizing substances do. On the basis of this assessment, the substance is not an oxidizer.

No classification for oxidising properties is proposed for PHMB.

7 ENVIRONMENTAL HAZARD ASSESSMENT

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

7.1.1.1 Fish

Short-term toxicity to fish

Only one study, considered as the most sensitive and validated by RMS (reliability factor = 1), has been reported (**Penwell and Roberts, 1996**). This test was performed according to EPA Standard Evaluation Procedure 540/9-85-006 and with radiolabelled PHMB. The 96-h LC_{50} and NOEC in a flow-through study for rainbow trout (*Oncorhynchus mykiss*), are respectively 26 µg l^{-1} and 9.8 µg l^{-1}, expressed in PHMB measured concentrations.

Long-term toxicity to fish

The effects on the growth of juvenile rainbow trout (*Oncorhynchus mykiss*) were examined in a study conducted according to OECD 215 (reliability factor = 1) (**Penwell and Roberts, 2001**). Sixteen individually branded fish per vessel were exposed to mean measured concentrations of ^{14}C-PHMB at 0, 1.0, 1.7, 3.0, 5.5, 10.0, 17.0 and 32.0µg l^{-1}. The test was carried out as a flow-through system over a 28-day duration, and the effects on growth were determined by a calculation of the relative (RGR, in g/g/day) and specific growth rate (SGR, % weight increase per day) rates, condition index and food conversion efficiency for different periods, 0 to 14, 14 to 28 and 0 to 28 days. The mean values of RGR and SGR for each exposure concentration were subjected to one-way analysis of variance and were compared with the dilution water control data using Dunnett's test, to determine any significance difference. For both, relative growth rate (RGR) and specific growth rate (SGR), the mean measured no observed effect PHMB concentration (NOEC) was 10 µg l^{-1} and the lowest observed effect concentration (LOEC) was 17 µg l^{-1}. The mean measured NOEC for both condition index and food conversion efficiency was 10 µg PHMB l^{-1} and the LOEC was 17 µg PHMB l^{-1}.

7.1.1.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

The only one acute study with *Daphnia magna* (**Brown and Pearson, 1981**), conducted prior to guideline publications but using a test protocol similar to OECD 202, could not be considered as valid (reliability factor = 3) due to important waiving (no GLP, no medium composition and no dissolved oxygen rate were reported, no reference substance tested and no PHMB monitoring during the test).

Long-term toxicity to aquatic invertebrates

A chronic toxicity study to *Daphnia magna* was conducted according to OECD 211 (**Penwell, 2006**), (reliability factor = 1). The test system was run over 21 days as a semi-static system, with replacements of test solutions every 2 days. 10 single parent animal replicates were deployed at test

concentrations of ^{14}C-PHMB at 0, 0.24, 0.8, 2.6, 8.4 and 24 µg l^{-1} (arithmetic mean measured concentrations). Offspring reproduction was counted throughout the test, and parent *Daphnia* length was measured after 21 days. For both test parameters, there were no significant differences from the control except at the highest concentration, 24 µg PHMB l^{-1}. Therefore, based on the mean measured concentrations of PHMB, the NOEC was 8.4 µg l^{-1} and the LOEC 24 µg l^{-1}.

7.1.1.3 Algae and aquatic plants

The PHMB toxicity towards the freshwater green algae, *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*), was performed according to OECD 201 guideline (reliability factor = 2) (**Penwell and Smyth, 2006**). Due to PHMB adsorption onto glassware, solutions of culture medium contaminated with [^{14}C]PHMB were prepared 24h before the beginning of the experiment corresponding with algal inoculation. [^{14}C]PHMB was at –24, 0 and 72 h of exposure. The maximum adsorption was observed between –24 and 0 h and the low loss of PHMB observed between 0 and 72 h could be due to a weak desorption of PHMB from glassware occurring by the increase of orbital agitation to homogenize the algal inocula. Therefore, measured concentrations at the end of the test are considered more realistic and the endpoints were calculated with these data. A Weibull curve has been plotted and a 72-h EC_{50} and NOEC were evaluated. According to TGD, NOEC is considered as EC_{10}.and 72-h ErC_{10} is 8 [7.3; 8.6] µg l^{-1} PHMB and 72-h ErC_{50} 15 [13.8; 16.8] µg l^{-1}.

7.1.1.4 Sediment organisms

The effects of ^{14}C-PHMB on sediment dwelling organisms were assessed in a study conducted according to OECD 218 (**Gilbert and Roberts, 2002a**), (reliability factor = 2). The larvae of *Chironomus riparius* were exposed to sediment spiked with PHMB at nominal concentrations of 100, 180, 320, 560 and 1000 mg kg^{-1} dry weight (the upper concentration being the limit concentration for studies of this type), and the PHMB concentrations were measured in overlying water, in the sediment and in pore water at the start and the end of the exposure. The mean actual concentrations of PHMB were 0, 73, 140, 240, 420 or 900 mg kg^{-1} dry weight. The daily emergence of adult midges monitored and assessed over a 28-day period. No effects were seen at any concentration, so the NOEC was estimated to be 900 mg kg^{-1} dry weight (corresponding with 196 mg.kg^{-1} wet weight) and the LOEC >900 mg kg^{-1} dry weight.

7.1.1.5 Other aquatic organisms

No data available.

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant for this type of dossier.

7.2 Terrestrial compartment

7.2.1 Toxicity test results

7.2.1.1 Toxicity to soil macro organisms

Not relevant for this type of dossier.

7.2.1.2 Toxicity to terrestrial plants

Not relevant for this type of dossier.

7.2.1.3 Toxicity to soil micro-organisms

No data available.

7.2.1.4 Toxicity to other terrestrial organisms

No data available.

7.2.2 Calculation of Predicted No Effect Concentration (PNEC_soil)

Not relevant for this type of dossier.

7.3 Atmospheric compartment

Not relevant.

7.4 Microbiological activity in sewage treatment systems

7.4.1 Toxicity to aquatic micro-organisms

In a study (**Penwell A.J & Roberts G.C, 2000a,** R=1) conducted according to ISO 9509: 1989 (E) Water quality – method for assessing the inhibition of nitrification of activated sludge microorganisms by chemicals and waste water - , the effect of PHMB upon the inhibition of nitrification was assessed. The test measured the inhibitory effects of nominal concentrations of PHMB at 5, 10, 20, 50 and 100 mg l^{-1} on nitrifying bacteria in activated sludge which, through the process of nitrification, oxidise ammonium salts in solution to nitrite and nitrate. The degree of inhibition was measured by assessing the difference in concentration of oxidized nitrogen (nitrite plus nitrate) produced by the oxidation of ammonium salts after parallel aeration of a nitrifying sludge in the presence and absence of the test substance over a four hour period.

A reference substance known to inhibit nitrification, 1-allyl-2-thiourea, was used. Flasks were incubated at 20 ± 2°C in a shaking incubator for 4 hours. At the end of the incubation period, the oxidised nitrogen formed in each test flask was calculated by subtraction of the mean value measured in the duplicate control flasks at the start of the test. RMS considered as valid without restrictions (**R = 1).** The results showed the **EC_{50} to be 38 mg PHMB l^{-1} with a NOEC of 12 mg PHMB l^{-1}**.

A second study (**Penwell A.J & Roberts G.C, 2000b,** R=1) was performed according to ISO/DIS draft 13641-1, water quality – determination of the inhibition of the activity of anaerobic bacteria **(R = 1**). The effect of PHMB upon the anaerobic gas production from sewage sludge organisms as typically found in domestic sewage treatment was determined. Nominal PHMB concentrations of 1, 2, 4, 7.5, 15, 30, 60, 125, 250 and 500 g l^{-1} were incubated with anaerobic sludge over 48 h. The inhibitory effect upon the production of CO_2 was measured by assessing the difference in amount of gas produced by the sludge microorganisms after parallel incubation of anaerobic sludge in both the presence and absence of PHMB over the 48 h period.

The EC_{50} was found to be 2.4 g PHMB l^{-1} (equivalent to 86 mg PHMB g^{-1} MLTS) and the **NOEC was 0.56 g PHMB l^{-1}** (equivalent to 20 mg PHMB g^{-1} MLTS).

7.4.2 PNEC for sewage treatment plant

Not relevant for this type of dossier.

7.4.3 Conclusion on the environmental classification and labelling

Data are summarised in Table 26 below.

Table 26 Summary of acute and long term toxicity of PHMB to the most sensitive species within different groups of aquatic organisms

Organism	Species	Test conditions	LC_{50}/ EC_{50}	NOEC (µg/L)	GLP (Y/N)	Reliability
Fish	*Oncorhynchus mykiss* (Rainbow trout)	96 h flow-through/Freshwater	26 µg l^{-1} (mean measured)	9.8 µg l^{-1} (mean measured)	Y	1
	Oncorhynchus mykiss (Rainbow trout)	Growth rate of juvenile fish, flow-through, 28 days/Freshwater		10 µg l^{-1} (mean measured)	Y	1
Invertebrates	*Daphnia magna* (waterflea)	48h, static	90 µg l^{-1} (nominal)	< 20 µg l^{-1} (nominal)	N	3
	Daphnia magna (waterflea)	Growth and reproduction, semi-static, 21 days		8.4 µg l^{-1} (mean measured)	Y	1
Algae	*Pseudokirchneriella subcapitata (formerly known as Selenastrum capricornutum)*	Static, 72h/Freshwater Biomass: Growth rate:	 11.4 [10.6;12.3] µg l^{-1} (mean measured) 15 [13.8;16.8] µg l^{-1} (mean measured)	 4.6 [4.3; 4.9] µg l^{-1} (mean measured) 8.0 [7.3; 8.6] µg l^{-1} (mean measured)	Y	1
Sediment dweller	*Chironomus riparius*	28 d, emergence of adult midges/ spiked sediment		196 mg kg^{-1} wet weight sediment (mean measured)	Y	2

The LC_{50} and EC_{50} values for fish, invertebrates and algae, are lower than 1 mg l^{-1} and although the acute toxicity data for Daphnia (reliability 3) are not reliable, it can be concluded that crustaceans are not more sensitive than fish and algae, based on the other available data. In addition, PHMB is not rapidly biodegradable, is photolytically and hydrolytically stable, and is expected to be persistent. However, based on PHMB physico-chemical properties (polymer, MW > 700Da, log Kow < 3), it is unlikely for the substance to bioaccumulate.
Therefore, **N; R50/53** is proposed according to Directive 67/548/EEC criteria.

Based on the CLP criteria, the proposed classification is Aquatic Acute 1 – H400 and Aquatic Chronic 1 – H410 with signal word "*Danger*" and pictogram GHS09.

In addition, as the 72h-EC_{50} value for algae is 0.01 mg l^{-1} < EC_{50} ≤ 0.1 mg l^{-1}, a M-factor of 10 is thus proposed to determine the specific concentration limit.

For the same reason, SCL are proposed for environment under Directive 67/548/EEC:

Specific concentration limits

C ≥ 2.5%	N, R50/53
0.25% ≤ C ≤ 2.5%	N, R51/53
0.025% ≤ C ≤ 0.25%	R52/53

Based on the CLP criteria, following the 2nd ATP, the substance should also be classified as Category Acute 1 – H400 (EC_{50} ≤ 1 mg l^{-1}) and Category Chronic 1-H410 (Non-rapidly degradable substances for which there are adequate chronic toxicity data available and with a chronic NOEC ≤ 0.1mg l^{-1}) with an M-factor of 10 for acute toxicity.

In addition, since the substance is not rapidly biodegradable and the lowest NOEC value ≤0.01 mg l^{-1} (NOEC algae = 0.008 mg l^{-1}), an M-factor of 10 should be applied for chronic toxicity.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

PHMB is currently under evaluation by the Rapporteur Member State France in the context of the Biocidal Product Directive (98/8/EC). In accordance with Article 36(2) of the CLP Regulation, PHMB should be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental end points.

OTHER INFORMATION

The information available here was submitted in the scope of the Biocidal Product Directive for inclusion of the active substance PHMB in annex I of directive 98/8/CE.

REFERENCES

Bannon C.J. 2008. MELTING POINT OF SOLID PHMB. Report 122-08B10PHMB.

Berry DF, Gore CW, Iswaren TJ, Phillips CE, Weight TM. 1977. Polyhexamethylene biguanide: Long term feeding study in the rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK Report No: CTL/P/333. July 27, 1977.

Blake J. 2003. Product Chemistry and Phys/chemical characteristics study for EPA, Grangemouth solid PHMB. (By analysis of chemical structure and not by experimentation) Analytical Science Group, Blackley, Manchester, UK Project 1273537.

Brammer, A. 1993. Polyhexamethylene Biguanide PHMB: Development Toxicity Study in the Rabbit. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/T/3997.

Bowhill L. 2007. PHMB: Determination of n-Octanol:Water Partition Coefficient. InterTek Analytical Science Group, Blackley, Manchester, UK. Study 1304881.

Brown E.A. 2002. Historical control data for occurrence of hemangiosarcoma (angiosarcoma) in C57BL/10J/Alpk BABU mice at Syngenta Central Toxicology Laboratory Alderley Park Macclesfield, Cheshire, UK. Avecia Inc. Report No. AP-1

Brown D., Pearson C.R. 1981. Acute toxicity of Vantocil IB to Daphnia magna. Imperial Chemical Industries Ltd., Brixham Laboratory. Report no. BLS/B/0041.

Busey, W. M. 1996. Polyhexamethylene Biguanide: Two Year Feeding Study in rats – Pathology Working Group Peer Review of Proliferative Vascular Lesions in Male and Female Rats. Central Toxicology Laboratory (CTL) Study Number PR0936 and Experimental Pathology Laboratories, Inc. (EPL) Project Number 371-002. EPL, Research Triangle Park, NC. CTL/C/3172.

Callander, R. D. 1989. Vantocil IB (PHMB): An evaluation in the Salmonella Mutation Assay. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/2406.

Carney, I. F., Gore, C.W., Iswran, T.J., Murphy, J.A., Riley, R.A. 1976. Vantocil IB: Sub acute inhalation study. Imperial Chemical Industries Limited, division reference no. ORG/29/75. Report number CTL/T/983.

Chang S.I. 2008. Determination of the Vapour Pressure of Poly(hexamethylene) Biguanide (PHMB).Report CASR-03-2008

Clapp, M.J L., Iswaran TJ, Rowson SM, Major TM. 1977a Polyhexamethylene biguanide: Life-time feeding study in the mouse. Central Toxicology Laboratory, Alderley Park Macclesfield, Cheshire, UK Report No: CTL/P/332.July 7, 1977

Clapp, M. J. L., T. J. Iswaran, and P. Major. 1977b. Polyhexamethylene Biguanide: 80-Week Skin Painting Study in Mice. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/331.

Clowes, H. M. 1996. PHMB: In Vitro Absorption through Human Epidermis. Central Toxicological Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/5120.

Clowes, H. M. 1998. PHMB: In Vitro Absorption through Human Epidermis at Spa Temperature. Central Toxicological Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/5916.

DeMatteo VA. 2008. Study protocol: Preliminary analysis of technical grade Polyhexamethylene biguanide (PHMB). 120-08B10PHMB.

Driscoll, R. 2003a. Acute Oral Toxicity in the Rat – Up and Down Procedure using PHMB. SafePharm Laboratories, Ltd. Shardlow Business Park, London Road, Shardlow, Derbyshire, England. Report #780/273.

Driscoll, R. 2003b. Acute Eye Irritation in the Rabbit. SafePharm Laboratories, Ltd. Shardlow Business Park, London Road, Shardlow, Derbyshire, England. Report # 780/276.

Driscoll, R. 2003c. Acute Dermal Toxicity (Limit Test) in the Rat. SafePharm Laboratories, Ltd. Shardlow Business Park, London Road, Shardlow, Derbyshire, England. Report # 780/274.

Driscoll, R. 2003d. Acute Dermal Irritation in the Rabbit. SafePharm Laboratories, Ltd. Shardlow Business Park, London Road, Shardlow, Derbyshire, England. Report # 780/276.

Duerden, L. 1993. Skin sensitisation to the guinea pig of a 20% aqueous solution of PHMB. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/3889.

ECHA, 2008. Guidance on information requirements and chemical safety assessment.

Field B.P. 1991. VANTOCIL P: Measurement of selected physical/chemical properties. Analytical Science Group, Blackley, Manchester, UK. Project 0176.

Foretz M, Hébrard S, Leclerc J, Zarrinpashneh E, Soty M, Mithieux G, Sakamoto K, Andreelli F, Benoit Viollet B 2010. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPH pathway via a decrease in hepatic energy state. J. Clin. Invest. 2010; 120(7):2355

Gilbert J.L., Roberts G.C. 2002a. PHMB: Toxicity to the sediment dwelling larvae Chironomus riparius. AstraZeneca UK Ltd., Brixham Environmental Laboratory. Report no. BL7135/B.

Gilbert J.L., Roberts G.C. 2002b. PHMB: Acute toxicity to the earthworm Eisenia foetida. AstraZeneca UK Ltd., Brixham Environmental Laboratory. Report no. BL7134/B.

Gilbert J L, Roberts G C, Woods C B. 1995. PHMB: activated sludge sorption and desorption, AstraZeneca Limited, Brixham Environmental Laboratory, Report no. BL5385/B.

Hink, G. 1976. Baquacil SB (PHMB): Photo-reaction Patch Test Using Natural Sunlight. Hill Top Research, Ohio, USA. Report No.: CTL/C/2163.

Hodge, M. C. E. and S. Palmer. 1976. Baquacil SB: A Teratology Study in the Rat by Dietary Administration. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/262.

Horner, S. A. 1995. Polyhexamethylene Biguanide: 1-Year Dietary Toxicity Study in the Dog. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/4488.

Horner, S. A. 1996. Polyhexamethylene Biguanide: Two-Year Feeding Study in Rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/4663.

Howard, C. 1989. Vantocil IB (PHMB): An Evaluation in the in vitro Cytogenetic Assay in Human Lymphocytes. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/2582.

Jackson, S. J. 1979. Vantocil P: Acute Oral and Dermal Toxicity in the Rat and Rabbit. Central Toxicological Laboratory, Alderley Park, Macclesfield, Cheshire, UK Report No.: CTL/T/1361.

Jackson, S. J. 1980a. Vantocil P: Skin Irritation Study in the Rabbit. Central Toxicological Laboratory, Alderley Park, Macclesfield, Cheshire, UK Report No.: CTL/T/1409.

Jackson, S. J. 1980b. Vantocil IB: Skin Sensitisation Studies. Central Toxicological Laboratory, Alderley Park, Macclesfield, Cheshire, UK Report No.: CTL/T/1423.

Jackson, S. J. and Oliver, G.J.A. 1983a. Vantocil IB: The effect of variation in induction concentration on skin sensitization in the guinea pig. Central Toxicological Laboratory, Alderley Park, Macclesfield, Cheshire, UK Report No.: CTL/T/1952.

Jackson, S. J. 1983b. Vantocil IB and chlorohexidine gluconate: potential for cross-reactivity in a skin sensitization study. Central Toxicological Laboratory, Alderley Park, Macclesfield, Cheshire, UK Report No.: CTL/T/1953.

Kamendulis, L. M. 2008. Studies to Elucidate the Potential Involvement of the Kupffer Cell in PHMB Mouse Liver Hemangiosarcomas. Department of Pharmacology and Toxicology. Indiana University School of Medicine. Indianapolis, Indiana.

Kilgour J.D. 1999. “BERMUDA”: 4 hour acute inhalation toxicity study in rats Central Toxicological Laboratory, Macclesfield, UK. Report No CTLP/6316.

Lees , D. and A. M. Leah. 1993. 21-Day Dermal Toxicity Study in the Rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/4200.

Long K W J, Roberts GC. 1994. PHMB: Aerobic biodegradation in water. Zeneca Ltd., Brixham Environmental Laboratory. Report no. BL5172/B.

Lythgoe, RE, Howard EF and Prescott E.. 1995a. PHMB: Absorption, Distribution, Metabolism and Excretion following Single Oral Dosing (20 mg/kg) in the Rat. Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK. Report No. CTL/P/4537.

Lythgoe, RE. and Howard EF. 1995b. PHMB: Bioavailability following Dietary Administration in the Rat. Central Toxicological Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/4595.

MacLean S.A., Palmer S., Penwell A.J., Roberts G.C. 2005b. PHMB: Aerobic sewage treatment simulation and chronic toxicity of treated effluent to Daphnia magna, AstraZeneca UK Limited., Brixham Environmental Laboratory. Report no. BL7802/B.

MacLean S.A., Penwell A.J., Roberts G.C. 2005a. PHMB: Biodegradability in seawater, AstraZeneca Limited, Brixham Environmental Laboratory, Report no. BL7804/B.

Macnab J.I. 2008. Determination of the vapour pressure of poly(hexamethylene)biguanide. Syngenta Technology and Projects Process Hazards Section, Huddersfield, UK PC/274.

Mann, P. 2002. Polyhexamethylene Biguanide: Two Year Oncogenic Study in Mice – Pathology Working Group Peer Review of Proliferative Vascular Lesions in Male and Female Mice. Central Toxicology Laboratory (CTL) Study Number PM0397 and Experimental Pathology Laboratories, Inc. (EPL) Project Number 698-001. EPL, Research Triangle Park, NC.

McFadden J P, Wakelin S, Holloway D B, Rycroft R J G, White I R, Basketter D A. 1998. Positive patch test reactions to polyhexamethylene biguanide. Abstract 5th Congress of the European Society of Contact Dermatitis, Helsinki.

Milburn, G. M. 1995. Polyhexamethylene Biguanide (PHMB): Multigeneration Study in the Rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/4455.

Milburn, G. M. 1996. Polyhexamethylene Biguanide: Two Year Oncogenic Study in Mice. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/4649.

Musi N, Hirshman MF, Nygren J et al. Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. Diabetes 2002; 51: 2074-2081.

Noakes, J. P. 2006. Polyhexamethylene Biguanide: 28-Day Inhalation Study in the Rat with Recovery. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/MR0219.

O'Malley L.P., Collins A.N., White G.F. 2006. Biodegradability of end-groups of the biocide polyhexamethylene biguanide (PHMB) assessed using model compounds. J Ind Microbiol Biotechnol 33: 677-684

O'Malley L.P., Shaw C.H., Collins A.N. 2007. Microbial degradation of the biocide polyhexamethylene biguanide: isolation and characterization of enrichment consortia and determination of degradation by measurement of stable isotope incorporation into DNA. Journal of Applied Microbiology 103 (4): 1158-1169

Penwell A.J. 2006. PHMB: Chronic toxicity to Daphnia magna. AstraZeneca UK Ltd., Brixham Environmental Laboratory. Report no. BL8365/B.

Penwell A.J., Roberts G.C. 1996. PHMB: Acute toxicity to rainbow trout Oncorhynchus mykiss. Zeneca Ltd., Brixham Environmental Laboratory. Report no. BL5506/B.

Penwell A.J., Roberts G.C. 2000a. VANTOCIL IB: Inhibition of nitrification of activated sludge microorganisms, AstraZeneca Limited, Brixham Environmental Laboratory. Report no. BL6913/B (unpublished).

Penwell A.J., Roberts G.C. 2000b. VANTOCIL IB Inhibition of anaerobic gas production from sewage sludge, AstraZeneca Limited, Brixham Environmental Laboratory. Report no. BL6914/B (unpublished).

Penwell A.J., Roberts G.C. 2001. PHMB: Effect on growth of juvenile rainbow trout Oncorhynchus mykiss. AstraZeneca UK Ltd., Brixham Environmental Laboratory. Report no. BL7096/B.

Penwell A.J., Roberts G.C. 2002. PHMB: Effect on seedling emergence and growth. AstraZeneca UK Ltd., Brixham Environmental Laboratory. Report no. BL7131/B.

Penwell A.J., Smyth D.V. 2006. PHMB: Toxicity to the green alga Selenastrum capricornutum. AstraZeneca UK Ltd., Brixham Environmental Laboratory. Report number BL8161/B,

Randall, V and S. L. Beck. 1989. Vantocil IB (PHMB): An Evaluation in the Mouse Bone Marrow Micronucleus Test. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/2436.

Richeux, F. 2002a. Assessment of acute dermal toxicity in rats. Phycher Bio développement, laboratory report no. TAD-PH-02/0013.

Richeux, F. 2002 Assessment of acute irritant/corrosive effect on the skin. Phycher Bio développement, laboratory report no. IC-OCDE-PH-02/0013.

Richeux, F. 2008a. Skin irritation test in the rabbit. Phycher Bio développement, laboratory report no. IC-OCDE-PH-08/0131

Richeux, F. 2008b. Eye irritation test in the rabbit. Phycher Bio développement, laboratory report no. IO-OCDE-PH-08/0131

Richeux, F. 2002c. Assessment of sensitising properties on albino guinea pig: Maximisation test according to Magnusson & Kligman. Phycher Bio développement, laboratory report no. SMK-PH-02/0013.

Sabljic A. and Güsten H. 1995. QSARs for soil sorption. In: Overview of Structure-Activity Relationships for Environmental Endpoints. Hermens JLM (ed.), Report prepared within the framework of the project "QSAR for Prediction of Fate and Effects of Chemicals in the Environment", an international project of the Environmental Technologies RTD Programme (DG XII/D-1) of the European Commission under contract number EV5V-CT92-0211.

Schofield D.J. 2007. Vantocil 100: Physical Chemical Testing. InterTek Analytical Science Group, Blackley, Manchester, UK. Study 1307428.

Schnuch A. *et al*. 2000. Polyhexamethylene biguanide: a relevant contact allergen? Contact Dermatitis. 42; 302

Schnuch A. *et al.* 2007. The biocide polyhexamethylene biguanide remains an uncommon contact allergen. Contact Dermatitis. 56; 235-239

Sielken R.L, Valdez-Flores C and Bretzlaff R. S. 2010. Statistical review of Polyhexamethylene Biguanide (PHMB):Carcinogenicity Studies, Pathology working groups, scientific advisory panel report and regulatory responses. 19 October 2010 (unpublished).

Smith, I. 1981. Vantocil IB (PHMB): Human sensitisation testing of Vantocil IB. Central Toxicology Laboratory, Macclesfield, UK. Report No.: CTL/C/1109.

Sudworth J. 2002. DS6222: Physico-Chemical Data- Project 1270585 Analytical Science Group, Blackley, Manchester, UK Project 1270585.

Sudworth J. 2006. PHMB: hydrolysis as a function of pH, InterTeK ASG, Backley, Manchester, UK, Project 1302832.

Sudworth J. 2002. Physico-chemical data. Avecia Ltd., Backley, Manchester, UK, Project 1270585.

Sueki, H. 2001. Polyhexamethylene Biguanide Cosmocil CQ: Skin Irritation in Humans. Department of Biochemical Toxicology and Department of Dermatology, Showa University, Japan. Report no. APJ-1.

Trutter, J.A. and Reno, F.E.. 1977. 20% PHMB: 3-generation reproduction study in rats, Hazleton Laboratories America, Inc., laboratory report no. not stated

Trueman, R. W. 1989. Vantocil IB (PHMB): Assessment for the Induction of Unscheduled DNA Synthesis in Rat Hepatocytes in vivo. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No: CTL/P/2603.

US EPA Cancer Assessment Review Committee. Cancer Assessment Document. Evaluation of the carcinogenic potential of PHMB. PC CODE 111801. July 16, 2003