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

EC number: not allocated (polymer)
CAS number: 27083-27-8 or 32289-58-0

CLH-O-0000003799-56-03/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

14 March 2014

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Polyhexamethylene biguanide or Poly(hexamethylene) biguanide hydrochloride or PHMB

EC Number: not allocated (polymer)

CAS Number: 27083-27-8 or 32289-58-0

Index Number : none

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance 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
Annex VI Index number:	None
Degree of purity:	> 94.2% w/w in dry weight
Impurities:	Information on impurities is confidential and then provided in a confidential part of the dossier in appendix 1.

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	No current Annex VI entry but an opinion was issued by the RAC on 9 September 2011: Acute Tox. 4 – H302 Acute Tox. 1 – H330 Eye Damage 1 – H318 Skin Sens. 1B – H317 STOT RE 1 – H372 (respiratory tract) (inhalation) Carc 2 – H351	No current Annex VI entry but an opinion was issued by the RAC on 9 September 2011: Xn; R22 T+; R26 Xi; R41 R43 T; R48/23 Carc. Cat. 3; R40

	Aquatic Acute 1 - H400, M=10 Aquatic Chronic 1 - H410, M=10	N, R50/53*
Current proposal for consideration by RAC	Acute Tox 2 – H330	T; R23
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox 4 – H302 Acute Tox 2 – H330 Eye Damage 1 – H318 Skin Sens 1B – H317 STOT RE 1 – H372 (respiratory tract) (inhalation) Carc 2 – H351 Aquatic Acute 1 - H400, M=10 Aquatic Chronic 1 - H410, M=10	Xn; R22 T; R23 Xi; R41 R43 T; R48/23 Carc. Cat. 3; R40 N, R50/53*

* specific concentration limits : N; R50/53: $C \geq 2.5\%$; N, R51/53: $0.25\% \leq C \leq 2.5\%$; R52/53: $0.025\% \leq C \leq 0.25\%$

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not in the scope of the present proposal		None	Conclusive but not sufficient for classification
2.2.	Flammable gases				
2.3.	Flammable aerosols				
2.4.	Oxidising gases				
2.5.	Gases under pressure				
2.6.	Flammable liquids				
2.7.	Flammable solids			None	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures				
2.9.	Pyrophoric liquids				
2.10.	Pyrophoric solids				
2.11.	Self-heating substances and mixtures				
2.12.	Substances and mixtures which in contact with water emit flammable gases				
2.13.	Oxidising liquids				
2.14.	Oxidising solids			None	Conclusive but not sufficient for classification
2.15.	Organic peroxides				
2.16.	Substance and mixtures corrosive to metals				
3.1.	Acute toxicity - oral			Acute Tox. 4*	
	Acute toxicity - dermal			None	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	Acute Tox. 2	Not relevant	Acute Tox. 1*	-
3.2.	Skin corrosion / irritation	Not in the scope of the present proposal		None	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation			Eye Damage 1*	
3.4.	Respiratory sensitisation			None	Data lacking
3.4.	Skin sensitisation			Skin Sens. 1B*	
3.5.	Germ cell mutagenicity			None	Conclusive but not sufficient for

					classification
3.6.	Carcinogenicity			Carc 2*	
3.7.	Reproductive toxicity			None	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure			None	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure			STOT RE 1*	
3.10.	Aspiration hazard			None	Conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment			Aquatic Acute 1*	
5.1.	Hazardous to the ozone layer			Aquatic Chronic 1*	

* RAC opinion of 9 September 2011

Labelling: Signal word: Dgr
Hazard statements: H351, H330, H372, H302, H318, H317, H400, H410
Precautionary statements: not harmonised

Proposed notes assigned to an entry: none

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	Not in the scope of the present proposal		None	Conclusive but not sufficient for classification
Oxidising properties			None	Conclusive but not sufficient for classification
Flammability			None	Conclusive but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*				
Thermal stability				
Acute toxicity	T; R23 Oral acute toxicity not in the scope of the present proposal.	None	T+; R26* Xn; R22*	
Acute toxicity – irreversible damage after single exposure	Not in the scope of the present proposal		None	Conclusive but not sufficient for classification
Repeated dose toxicity			T; R48/23*	
Irritation / Corrosion			Xi; R41*	
Sensitisation			Xi; R43*	
Carcinogenicity			Carc. cat. 3; R40*	
Mutagenicity – Genetic toxicity			None*	Conclusive but not sufficient for classification
Toxicity to reproduction – fertility			None*	Conclusive but not sufficient for classification
Toxicity to reproduction – development			None*	Conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation			None*	Conclusive but not sufficient for classification
Environment			N; R50/53*	

* RAC opinion of 9 September 2011

Labelling: Indication of danger: T; N
R-phrases: R : 22-23-40-41-43-48/23-50/53
S-phrases: S : 22-26-36/37/39-45-60-61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

PHMB is currently not classified according to Annex VI of CLP.

PHMB is an active substance in the meaning of Directive 98/8/EC and shall be subject to harmonised classification and labeling for all hazard classes. A classification proposal considering all human health and environmental endpoints was submitted in 2009 and RAC issued an opinion on the recommended harmonised classification of PHMB on 9 September 2011.

In 2012, a new study on acute toxicity by inhalation has been made available and warrants to our opinion a change in the classification initially proposed and agreed by the RAC on this endpoint. The present CLH report therefore focuses on acute toxicity by inhalation.

Other endpoints are not in the scope of the present CLH report in absence of new information that may have changed their assessment since RAC opinion.

No registration dossier is available for PHMB.

2.2 Short summary of the scientific justification for the CLH proposal

The initial conclusion on classification Acute Tox 1 – H330 of PHMB for acute inhalation toxicity was based on the result of a 28-day inhalation study (Carney, 1976) that reported the death of all animals after a single exposure to PHMB aerosol but interpretation was limited by poor reporting. The validity of the results of this study was however supported by similar sensitivity in terms of NOAEC and LOAEC in another 28-day study performed according to OECD 412 (Noakes, 2006). The highest dose administered in Noakes (2006) was however 0.00247 mg/l and although no death was observed at this upper dose, it is of limited relevance to confirm or contradict the LC_{50} observed in Carney (1976).

RAC also considered in its analysis of 2011 the acute inhalation toxicity study by Kilgour (1999) on a formulation containing 20.6% of PHMB. In this study a LC_{50} higher than 0.36 mg/l PHMB was assumed. RAC came to the following conclusion:

> *"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 Acute Tox 1– H330 (CLP), and T+; R26 (DSD) is warranted based on the results from the study by Carney (1976)."*

The new study available (confidential reference 2012) is of good quality (GLP and according to the OECD guideline) and reports a LC_{50} of 0.29 mg/l in male rats. The study was performed in an aqueous vehicle but a difference in vehicle cannot explain the difference in results between this new study (2012) and Carney (1976) as also hypothesised to explain differences in results with Kilgour (1999).

Differences in rat strains used in the various studies exist but are unlikely to explain a difference in sensitivity of a factor of 10. It is also noted that although small and therefore involving a certain degree of variability, the number of animals used in the different studies are in line with guidelines except a slight difference in Carney (1976) where 4 animals/sex/dose were used instead of 5.

In absence of report that actual exposure concentrations have been controlled in Carney (1976), a doubt on the exact concentration to which animals were exposed is raised by the results obtained in the more recent studies.

Considering all the data now available and based on a weight of evidence, it is considered that the new 2012 study should be used as the relevant study for classification of PHMB for acute inhalation toxicity. **The critical LC_{50} is therefore 0.29 mg/l for male rats.**

On this basis, a classification Acute Tox 2 – H330 (CLP), and T; R23 (DSD) is warranted.

2.3 Current harmonised classification and labelling

No current harmonised classification in Annex VI of CLP but the following opinion was adopted by RAC on 9 September 2011:

	CLP classification	**Directive 67/548/EEC (Dangerous Substances Directive; DSD) classification**
Current entry in Annex VI, CLP Regulation	No current Annex VI entry but an opinion was issued by the RAC on 9 September 2011: Acute Tox. 4 – H302 Acute Tox. 1 – H330 Eye Damage 1 – H318 Skin Sens. 1B – H317 STOT RE 1 – H372 (respiratory tract) (inhalation) Carc 2 – H351 Aquatic Acute 1 - H400, M=10 Aquatic Chronic 1 - H410, M=10	No current Annex VI entry but an opinion was issued by the RAC on 9 September 2011: Xn; R22 T+; R26 Xi; R41 R43 T; R48/23 Carc. Cat. 3; R40 N, R50/53*

* specific concentration limits : N; R50/53: C ≥ 2.5% ; N, R51/53: 0.25% ≤ C ≤ 2.5% ; R52/53: 0.025% ≤ C ≤ 0.25%

2.4 Current self-classification and labelling

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
R43
N ; R50/53

The following classifications have been notified in the C&L inventory (5 notifications).

Acute toxicity by oral route	Acute Tox. 4 – H302 or no classification
Acute toxicity by inhalation	Acute Tox. 1 – H330 or no classification
STOT SE	STOT SE 3- H335 or no classification
Skin irritation/corrosion	Skin Irrit. 2 – H315
Eye irritation/corrosion	Eye Damage 1 – H318 or Eye Irrit. 2 – H319
Skin sensitisation	Skin Sens. 1 – H317
STOT RE	STOT RE 1 – H372 or no classification
Carcinogenicity	Carc 2 – H351 or no classification
Aquatic acute toxicity	Aquatic Acute 1 - H400 or no classification
Aquatic chronic toxicity	Aquatic Chronic 1 - H410 (with or without M=10)

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

PHMB is currently not classified according to Annex VI of CLP.

PHMB is an active substance in the meaning of Directive 98/8/EC. In accordance with Article 36(2) of the CLP Regulation, PHMB shall be subjected to harmonised classification and labeling for all hazard classes.

A classification proposal considering all human health and environmental endpoints has been submitted in 2009 and RAC issued an opinion on the recommended harmonised classification of PHMB on 9 September 2011.

In 2012, a new study on acute toxicity by inhalation has been made available and warrants to our opinion a change in the classification initially proposed and agreed by the RAC on this hazard class. The present CLH report therefore focuses on acute toxicity by inhalation.

The other endpoints are not in the scope of the present CLH report in absence of new information that may have changed their assessment since RAC opinion.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

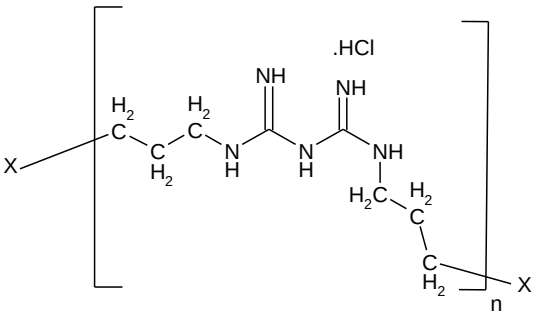
1 IDENTITY OF THE SUBSTANCE

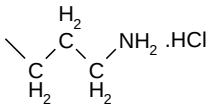
1.1 Name and other identifiers of the substance

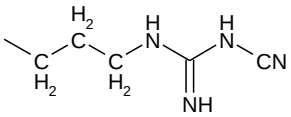
Table 5: Substance identity

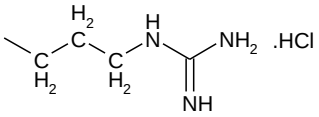
EC number:	Not allocated as the substance is a polymer (the substance was not notified under Directive 92/32/EEC)
EC name:	Not allocated as the substance is a polymer (the substance was not notified under Directive 92/32/EEC)
CAS number (EC inventory):	27083-27-8 and 32289-58-0
CAS number:	Two equivalent 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.
CAS name:	Polyhexamethylene biguanide hydrochloride
IUPAC name:	Polyhexamethylene biguanide hydrochloride
CLP Annex VI Index number:	None
Molecular formula:	$(C_8H_{17}N_5)_n.nHCl$, n=1-40
Molecular weight range:	See confidential data in appendix I

Structural formula:



Where X = 

Or X = 

Or X = 

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

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Polyhexamethylene biguanide hydrochloride	> 94.2% w/w in dry weight		

Current Annex VI entry:

No harmonised classification but the following opinion was adopted by RAC on 9 September 2011:

According to table 3.2	According to table 3.1
Xn; R22	Acute Tox. 4 – H302
T+; R26	Acute Tox. 1 – H330
Xi; R41	Eye Damage 1 – H318
R43	Skin Sens. 1B – H317
T; R48/23	STOT RE 1 – H372 (respiratory

Carc. Cat. 3; R40 N, R50/53*	tract) (inhalation) Carc 2 – H351 Aquatic Acute 1 - H400, M=10 Aquatic Chronic 1 - H410, M=10

* specific concentration limits : N; R50/53: C ≥ 2.5% ; N, R51/53: 0.25% ≤ C ≤ 2.5% ; R52/53: 0.025% ≤ C ≤ 0.25%

Impurities and additives are confidential and described in a confidential part of the dossier in appendix 1.

1.3 Physico-chemical properties

Table 7: Summary of physico - chemical properties

Property	Value	Reference
State of the substance at 20°C and 101,3 kPa	Off white to pale yellow powder with strong ammonia smell.	Sudworth, 2002
Melting/freezing point	78.9-136.3ºC	Bannon, 2008
Boiling point	The substance decomposes at 205-210°C before boiling	Field, 1991
Relative density	1.20 ± 0.0025 (20°C ± 0.5°C)	Sudworth, 2002
Vapour pressure	1.32×10^{-7} Pa (20°C) 4.11×10^{-7} Pa (25°C)	Chang, 2008
Surface tension	68.5 ± 0.6 mN/m temperature: 25 ± 0.5°C	Schofield, 2007
Water solubility	41% w/w ± 1% temperature: 25 ± 1°C	Sudworth, 2002
Partition coefficient n-octanol/water	Log P_{ow} = -2.3 (experimentally estimated) temperature: 25ºC ; pH: 7.4	Bowhill, 2007
Flash point		
Flammability	Not flammable	Schofield, 2007
Explosive properties	Not explosive	Schofield, 2007.
Self-ignition temperature	No Ignition Below 400°C (Upper Limit of Test)	Schofield, 2007.
Oxidising properties	Not oxidising	Schofield, 2007
Dissociation constant	pKa=4.19 at 25°C	Field, 1991

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

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

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not considered in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Not considered in this dossier.

4.2 Acute toxicity

Table 8: Summary table of relevant acute toxicity studies by inhalation

Method	Results	Remarks	Reference
28-day inhalation study (prior to GLP or guideline adoption)	LC_{50} < 0.030 mg/l	Alderley Park SPF albino rats n=4 /sex /dose Snout-only Test substance: aqueous solution of PHMB 20%	Carney, 1976
Acute toxicity study GLP, US EPA guideline 870-1300	LC_{50} > 0.36 mg/l	Alpk:APfSC rats n=5 /sex /dose Nose-only Test substance: 20% PHMB with unclear information on the non-active ingredients	Kilgour, 1999
Acute toxicity study GLP, OECD 403	LC_{50} males = 0.29 mg/l	Wistar CRL:(WI) rats n=5/sex /dose Nose-only Test substance: PHMB (purity 99.6%). Aerosol produced after dilution into water.	Confidential reference, 2012

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Not considered in this dossier.

4.2.1.2 Acute toxicity: inhalation

Data considered in the previous CLH report from FR:

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 section 4.7 for additional information on the study).

Alderley Park SPF albino rats (4/sex/group) were exposed snout-only 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.

In the high dose group (26 µg/L), 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 2.75 µg/L, where 3 males and 1 female died during or after the sixth exposure.

Based on this result, the LC_{50} can therefore be estimated, on the basis of limited available data, as smaller than 26 µg/L for a single 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.**

Additional data considered by the RAC on request of CEFIC in August 2011:

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.**

Additional data available since RAC's opinion of 9 September 2011:

Wistar CRL:(WI) rats (n=5/sex/concentration in the main study) were exposed nose-only to an aerosol of PHMB (purity 99.6%) in aqueous solution in a GLP, OECD 403-compliant acute inhalation toxicity study (**confidential reference, 2012**). Mass medium aerodynamic diameters were in the range of 1.49-2.20 µm with geometric standard deviation (GSD) in the range of 1.84-2.29 µm.

In the preliminary study, both animals exposed at 1.0 mg/L died after 1 and 2 hours of exposure respectively. Severely laboured respiration was observed and dark red, diffuse discoloration of enlarged or non collapsed lungs with foamy white content in trachea was observed at necropsy. At 0.1 mg/L, no lethality occurred, slight to moderate clinical signs were observed (laboured respiration, rhonchus, partial ptosis, decreased activity, increased respiratory rate, sneezing). Transient body weight decrease was observed but no test-item related macroscopic findings.

Exposure levels in the main study were 0.1, 0.3 and 0.5 mg/l PHMB for 4 hours.

Mortality is summarised in the table below.

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

Clinical signs

At a concentration of 0.1 mg/L, the main clinical signs were observed on Day 0 and included: slight to moderately laboured respiration (all males and 3 of 5 females), rhonchus (2 of 5 males and 3 of 5 female), decreased activity (all males), hunched back (4 of 5 females) and increased respiratory rate (all females). Following the exposure on Day 1, the respiratory signs ceased and slightly laboured respiration and/or rhonchus (noisy respiration) was noted in two males only, with sneezing observed in all males for 3-4 days (up to Day 5). In all males and in 2 of 5 females weak body condition was noted during Days 1 and 2.
In all animals exposed to a concentration of 0.3 mg/L, slight to moderately laboured respiration was noted during the exposure. Following the exposure on Day 0, severely laboured respiration with rhonchus was noted in 4 of 4 males. In all females, a moderately laboured respiration was accompanied by slightly increased respiratory rate and noisy respiration was noted for 3 of 5 females. Slight to severe decreased activity was observed in 4 of 4 males and in 4 of 5 females. In addition, in a single animal, partially closed eyelids and moderate ataxia were observed. One male which displayed severe signs like severe decreased activity and prostration on Day 0, had severe laboured respiration with gasping and rhonchus, decreased activity and ruffled coat before death in afternoon on Day 1. On the following days the clinical signs ceased in surviving males and only slightly laboured respiration, slightly increased respiratory rate and weak condition were observed up to Day 3. In females, weak body condition was noted in 4 of 5 females on Days 1 and 2. In addition in one female ruffled coat was observed on Days 2-3 and sneezing on Day 3.
At 0.5 mg/L the main clinical signs included: moderately to severely laboured respiration with noisy respiration up to gasping, increased respiratory rate and decreased activity. The clinical signs were observed following the exposure on Day 0. In two females, laboured respiration was observed up to Day 7. The respiration was moderately/severely laboured for 2-3 days after the exposure and in one animal accompanied by gasping and was noisy up to Day 6. In addition hunched back and weak condition were noted up to Day 6. Sneezing was observed up to necropsy on Day 14.

Effect on body weight

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

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

Necropsy

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

On the basis of this study, **LC_{50} were determined to be 0.29 mg/l for males, 0.48 mg/l for females and 0.37 mg/l for males and females combined** (1.85 mg/L for 20%-PHMB solution).

4.2.1.3 Acute toxicity: dermal

Not considered in this dossier.

4.2.1.4 Acute toxicity: other routes

Not considered in this dossier.

4.2.2 Human information

No data.

4.2.3 Summary and discussion of acute toxicity

The initial conclusion on classification Acute 1 – H330 of PHMB for acute inhalation toxicity was based on the result of a 28-day inhalation study (Carney, 1976) that reported the death of all animals after a single exposure to PHMB aerosol but interpretation was limited by poor reporting. The validity of the results of this study was however supported by similar sensitivity in terms of NOAEC and LOAEC in another 28-day study performed according to OECD 412 (Noakes, 2006). The highest dose administered in Noakes (2006) was however 0.00247 mg/l and although no death was observed at this upper dose, it is of limited relevance to confirm or contradict the LC_{50} observed in Carney (1976).

RAC also considered in its previous analysis of 2011 the acute inhalation toxicity study by Kilgour (1999) on a formulation containing 20.6% of PHMB. In this study a LC_{50} higher than 0.36 mg/l PHMB is assumed. RAC came to the following conclusion:

> *"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 Acute Tox 1– H330 (CLP), and T+; R26 (DSD) is warranted based on the results from the study by Carney (1976)."

The new study available is of good quality and reports a LC_{50} of 0.29 mg/l in male rats. The study was performed in an aqueous vehicle and a difference in vehicle cannot explain the difference in results between this study and Carney (1976) as hypothesised to explain differences in results with Kilgour 1999.

Differences in rat strains used in the various studies exist but are unlikely to explain a difference in sensitivity of a factor of 10. It is also noted that although small and therefore involving a certain degree of variability, the number of animals used in the different studies are in line with guidelines except a slight difference in Carney (1976) where 4 animals/sex/dose were used instead of 5.

In absence of report that actual exposure concentrations have been controlled in Carney (1976), some doubt on the exact concentration to which animals were exposed is raised by the results obtained in the more recent studies.

Considering all the data now available and based on a weight of evidence, it is considered that the new 2012 study should be used as the critical study for classification of PHMB for acute inhalation toxicity. **The critical LC_{50} is therefore 0.29 mg/l for male rats.**

4.2.4 Comparison with criteria

According to CLP, a classification Acute 2 – H330 applies when LC_{50} is higher than 0.05 mg/l and lower or equals to 0.5 mg/l for dust and mist.

According to DSD, a classification T; R23 applies when LC_{50} is higher than 0.25 mg/l and lower or equals to 1 mg/l for aerosols and particulates.

4.2.5 Conclusions on classification and labelling

On this basis, a classification Acute Tox 2 – H330 (CLP), and T; R23 (DSD) is warranted.

RAC evaluation of acute toxicity
Summary of the Dossier submitter's proposal The dossier submitter submitted information on previously considered studies on acute inhalation toxicity and on repeated dose toxicity, and in addition the results from a recently conducted acute inhalation study (confidential study report, 2012). The initial conclusion on classification of PHMB for acute inhalation toxicity (Acute Tox 1 – H330) was based on the results from a 28-day inhalation study (Carney, 1976). This study reported the death of all animals after a single exposure to PHMB aerosol and an LC_{50} of less than 0.03 mg/l was estimated for a 4 hour exposure), although interpretation of the study was limited by poor reporting. The validity of the results of this study was

however supported by the similar sensitivity in terms of NOAEC and LOAEC to another 28-day study performed according to OECD TG 412 (Noakes, 2006). The highest concentration administered in Noakes (2006) was however only 0.00247 mg/l and no deaths were observed at this concentration. The dossier submitter found this study to be of limited relevance in confirming or contradicting the LC_{50} value observed by Carney (1976).

The dossier submitter referred to the RAC analysis from 2011, which also considered the acute inhalation toxicity study by Kilgour (1999) using a formulation containing 20.6% PHMB. In this study an LC_{50} higher than 0.36 mg/l PHMB was assumed.

In 2011 RAC came to the following conclusion:

> *"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 Acute Tox 1– H330 (CLP), and T+; R26 (DSD) is warranted based on the results from the study by Carney (1976)."*

The dossier submitter stated that the new study available (confidential study report, 2012) was of good quality (GLP and according to the OECD guideline) and reported an LC_{50} of 0.29 mg/l in male rats. The study was performed with an aqueous vehicle, but according to the dossier submitter, differences in the vehicle could not explain the difference in the LC_{50} results between this new study and Carney (1976). Differences in the vehicle were also hypothesised to explain the difference between the results in the studies of Carney (1976) and Kilgour (1999).

The dossier submitter stated that differences in rat strains used in the various studies exist but that they were unlikely to explain a difference in sensitivity of a factor of 10. It was also noted that although a small number of animals was used in the different studies, which was likely to have contributed to the degree of variability, the numbers were in line with guidelines, apart from a slight deviation from the guideline in Carney (1976) where 4 animals/sex/concentration were used instead of 5.

In the absence of information on whether the actual exposure concentrations had been controlled in the study by Carney (1976), the dossier submitter considered that the results obtained in the more recent studies raised doubts regarding the exact concentrations to which animals were exposed (in the Carney study).

Taking into account all the currently available data and based on the weight of the evidence, the dossier submitter considered that the new 2012 study should be used as the relevant study for classification of PHMB for acute inhalation toxicity. The critical LC_{50} was therefore 0.29 mg/l for male rats.

On this basis, classification as Acute Tox 2 – H330 (CLP), was warranted.

Comments received during public consultation

Two member states supported the proposed classification as Acute Tox. 2, H330. A third member state requested more information on study details (purity of the test substance, concentration tested) and asked for explanations on the differences between previously considered studies and the new study.

Additional key elements

This overview on LC_{50} values from available single and repeated dose studies was presented by the dossier submitter (see RCOM document, Annex 2).

LC_{50} ("pure" PHMB)	**Study**	**Rat strain**	**Nber of rats/dose**	**Adminis-tration**	**Vehicle**
LC_{50} < 0.030 mg/l	Carney 1976 (28d)	Alderley Park SPF albino	n=4 /sex	Snout-only	Water
LC_{50} > 0.00247 mg/l	Noakes 2006 (28d)	Alpk:APfSD (Wistar-derived)	n=5 /sex	Nose-only	Water
LC_{50} > 0.36 mg/l	Kilgour 1999	Alpk:APfSC (Wistar-derived)	n=5 /sex	Nose-only	?
LC_{50} = 0.29 mg/l	Confidential reference, 2012	Wistar CRL:(WI)	n=5 /sex	Nose-only	Water

In the Kilgour study, the information on the vehicle/other ingredients in the test formulation containing 20.6% PHMB was lacking, no concentration higher than 0.36 mg/l was tested and no accurate LC_{50} could be calculated. The Noakes study did not examine higher concentrations than 0.00247 mg/l. Thus neither study contradicts the results of the new study. The lower LC_{50} values calculated from the Carney study could not be explained, as water was the vehicle in both the new study and in the Carney study and different rat strains were unlikely to be responsible for the different results. The dossier submitter found the new study to be the most reliable one.

Assessment and comparison with the classification criteria

In a recently submitted acute inhalation toxicity study (confidential study report, 2012), rats were exposed at concentrations of 0.1, 0.3 or 0.5 mg/l to an aerosol of PHMB (99.6%) in aqueous solution with particles with an MMAD range of 1.49-2.2 µm (GSD of ±1.84-2.29 µm). Clinical signs (laboured respiration, decreased activity, hunched posture, transient body weight loss on Day 1) were observed in male and female rats at all tested concentrations and the severity/incidence/duration of effects increased with the test concentration. Mortalities were observed at 0.3 mg/l and above. In this study, the LC_{50} values were determined to be 0.29 mg/l for males, 0.48 mg/l for females and 0.37 mg/l for males and females combined. These values are in the range of concentrations that justify classification for acute toxicity Category 2 for inhalation exposure to dust/mist (0.05 mg/l < Category 2 ≤ 0.5 mg/l).

RAC agrees with the assessment of the dossier submitter. The committee finds the new study robust, based on its compliance with OECD TG 403 and on its detailed description of clinical and lethality information.

RAC gives more weight to this study than to the less reliable single exposure study of Kilgour (1999) or to the repeated dose studies (Carney, 1976, Noakes, 2006). It is noted that differences in calculated LC_{50} values among all available studies could still not be fully explained. The previous RAC opinion was mainly based on the acute mortalities in the repeated dose studies due to the lack of robust single exposure studies. The discrepancies in the LC_{50} values among the less valid studies and the new study were not considered to be important, since more weight was given to the new study which was consistent with the guideline (OECD TG 403).

In its previous opinion from September 2011, RAC recommended to classify PHMB with Acute Tox. 1; H330 (inhalation). Based on the new information, classification as Acute Tox. 2; H330 (inhalation), as suggested by the dossier submitter, is supported by RAC.

No other hazard endpoints were addressed for classification purposes.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not considered in this dossier.

4.4 Irritation

Not considered in this dossier.

4.5 Corrosivity

Not considered in this dossier.

4.6 Sensitisation

Not considered in this dossier.

4.7 Repeated dose toxicity

Not considered in this dossier.

Data on repeated toxicity by inhalation are summarised here for information purpose only in relation to the discussion on acute toxicity by 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.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not considered in this dossier.

4.9 Germ cell mutagenicity (Mutagenicity)

Not considered in this dossier.

4.10 Carcinogenicity

Not considered in this dossier.

4.11 Toxicity for reproduction

Not considered in this dossier.

4.12 Other effects

Not considered in this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not considered in this dossier.

6 OTHER INFORMATION

No registration dossier is available for PHMB.

7 REFERENCES

Bannon C.J. 2008. Melting point of solid PHMB. Report 122-08B10PHMB.

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

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

Confidential reference: XXX 2012. Final report. XXXXX. Laboratory: XXX. Study code : XXX. (see annex II for full reference)

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.

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

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.

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

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