Committee for Risk Assessment

RAC

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/F

Adopted

9 September 2011

Annankatu 18 | P.O. Box 400 | 00121 Helsinki | Finland
www.echa.eu | Tel.: + 358 9 68.61.80

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OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT COMMUNITY LEVEL

In accordance with Article 37 (4) of the Regulation (EC) No 1272/2008 (CLP Regulation), the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling of

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**

The proposal was submitted by ***France***
and received by RAC on ***31 March 2010.***

The proposed harmonised classification:

	CLP Regulation (EC) No 1272/2008	Directive 67/548/EEC (criteria)
Current entry in Annex VI CLP Regulation	No entry (Table 3.1)	No entry (Table 3.2)
Current proposal for consideration by RAC	Carc. 2; H351 Acute Tox. 1; H330 STOT RE 1; H372 Acute Tox. 4; H302 Eye Dam. 1; H318 Skin Sens. 1; H317 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Carc. Cat.3;R40 T+;R26 T;R48/23 Xn; R22 Xi; R41 Xi;R43 N; R50/53
Resulting harmonised classification (proposed future entry in Annex VI CLP Regulation)	Carc. 2; H351 Acute Tox. 1; H330 STOT RE 1; H372 Acute Tox. 4; H302 Eye Dam. 1; H318 Skin Sens. 1; H317 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Carc. Cat.3;R40 T+;R26 T;R48/23 Xn; R22 Xi; R41 Xi;R43 N; R50/53

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/consultations/harmonised_cl_en.asp*** on ***31.03.2010*** Parties concerned and MSCAs were invited to submit comments and contributions by ***14.05.2010***.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: ***Agnes Schulte***
Co-rapporteur, appointed by RAC: ***Karen van Malderen***

The opinion takes into account the comments of MSCAs and parties concerned provided in accordance with Article 37 (4) of the CLP Regulation.**]**

The RAC opinion on the proposed harmonised classification and labelling has been reached on ***9th September 2011,*** in accordance with Article 37 (4) of the CLP Regulation, giving parties concerned the opportunity to comment. Comments received are compiled in Annex 2

The RAC Opinion was adopted by ***simple majority;*** one RAC member expressed a minority position regarding the RAC assessment for carcinogenicity. The minority position, including its grounds, was made available in a separate document which has been published at the same time as the opinion.

OPINION OF RAC

The RAC adopted the opinion that ***PHMB*** should be classified and labelled as follows:

Classification & Labelling in accordance with the CLP Regulation

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
	Polyhexamethylene biguanide or Poly(hexamethylene) biguanide hydrochloride or PHMB	not allocated	27083-27-8 or 32289-58-0	Carc. 2 Acute Tox. 1 STOT RE 1 (respiratory tract, inhalation) Acute Tox. 4 Eye Dam. 1 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H351 H330 H372 H302 H318 H317 H400 H410	GHS05 GHS06 GHS08 GHS09 Dgr	H351 H330 H372 H302 H318 H317 H410		Acute M = 10; Chronic M = 10.	

Classification & Labelling in accordance with Directive 67/548/EEC

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
	Polyhexamethylene biguanide or Poly(hexamethylene) biguanide hydrochloride or PHMB	Not allocated	27083-27-8 or 32289-58-0	Carc. Cat 3 ; R40 Xn ; R 22 T+; R26 T; R48/23 Xi; R41 Xi; R43 N; R50/53	T+; N R: 22-26-41-43-48/23-40-50/53 S: 22-26-36/37/39-45-60-61	N; R50/53: C ≥ 2.5% N, R51/53: 0.25% ≤ C ≤ 2.5 R52/53: 0.025% ≤ C ≤ 0.25%	

SCIENTIFIC GROUNDS FOR THE OPINION

The RAC opinion relates only to those hazard classes that have been reviewed in the proposal for harmonised classification and labelling, as submitted by France.

Acute toxicity: inhalation

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.

In August 2011, CEFIC requested RAC to consider an acute inhalation study (Kilgour, 1999). In this study a formulation containing 20.6 (% w/w) PHMB (but with unclear information on the non-active ingredients), rats 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. At this dose, one male died (out of ten animals in total). 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 Acute Tox 1 – H330 (CLP), and T+; R26 (DSD) is warranted based on the results from the study by Carney (1976).

Acute toxicity: oral

Two studies by oral route indicate a moderate acute toxicity with LD_{50} between 500 and 1000 mg/kg in rats. The RAC concludes that PHMB meets the criteria for classification in Acute Tox 4 – H302 (CLP Regulation; 300 mg/kg ≤ LD50 ≤ 2000 mg/kg) and as Xn; R 22 (DSD: 200 mg/kg ≤ LD50 ≤ 2000 mg/kg)

Acute toxicity: dermal

Two studies conducted at one single dose (400 mg/ kg and 5000 mg/kg) did not demonstrate signs of acute toxicity (guideline OECD 402). No classification is warranted for the dermal route.

Skin irritation

Three studies conducted according to guideline OECD 404 did not show evidence of skin irritation. Therefore, PHMB does not need to be classified for skin irritation.

Eye irritation

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 Eye damage 1 – H318 (CLP) and Xi; R41 (DSD) is warranted .

Skin sensitisation

Although no sensitisation was observed in repeat insult patch test (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 is 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 Skin Sens. 1 – H317 (CLP) and R43 (DSD) is therefore warranted.

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 (1993) and Richeux (2002c) 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.

Repeated dose toxicity: oral

Due to the absence of serious health effects in a rat study and at doses ≤25 mg/kg (corresponding to the upper guidance value of 100 mg/kg for a 90 day study) in a 12 month study in dogs there is no need to classify PHMB for this route of exposure.

Repeated dose toxicity: inhalation

Repeated dose toxicity of PHMB has been assessed by inhalation in two 28-day studies.

Repeated inhalation of PHMB aerosol 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, tracheitis, pneumonitis and bronchitis. Moderate to severe pneumonitis with areas of epithelial desquamation was reported at 2.75 µg/l. In one study with a 13-week recovery period, the lung lesions were not reversible. From 0.25µg/L, 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 2.75 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 justifies classification for acute inhalation toxicity. However, delayed mortalities were also observed after repeated exposure from doses two orders of magnitude lower, which justifies to considering 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 the 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 STOT RE 1 - H 372 (CLP) and T; R48/23 (DSD) is warranted.

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).

Repeated dose toxicity: dermal

Due to the lack of serious health effects at doses ≤ 60 mg/kg/day (guidance value for dermal 28-day studies) in a 21-day study in rats, there is no need to classify PHMB for this route of exposure.

Mutagenicity

Based on two *in vitro* studies (guidelines OECD 471 and 473) and two *in vivo* studies (guideline OECD 474 and UDS), PHMB is not considered as mutagenic and therefore there is no need to classify for this endpoint.

Carcinogenicity

After examination of the full study reports, RAC considers three carcinogenicity studies as appropriate basis for its conclusion, i.e. one oral study in mice, one oral study in rats and a dermal study in the mouse.

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 support 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 tumours 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.

- The oral rat study by Berry et al., 1977 was not considered acceptable by the dossier submitter for the CLH proposal and biocide CAR, since less than 50% of the animals were alive at the end of the 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, 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 hypoglycemic 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 says 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 Carc 2 – H351 (CLP) and category 3; R40 (DSD) 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.

Toxicity for reproduction

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). 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. Moreover, the effects seen on the weight male reproductive organs identified in the two-generation study were not related to histological changes and in some case dose-response, these effects were not considered of toxicological significance. Thus there is no need to classify PHMB for fertility.

No evidence of foetotoxicity and teratogenicity is observed in prenatal studies in the rat and the rabbit (guideline OECD 414). There is no need to classify PHMB for developmental toxicity.

Environment

All stakeholders who participated in the public consultation supported the proposed classification for the aquatic environment.

In a study performed according to the OECD guideline 111, less than 10% hydrolysis was found after 5 days for all pHs tested (pH 4, 7 and 9 at 50°C). Therefore PHMB is considered to be hydrolytically stable. No valid study concerning photolysis is available. Nevertheless, it was concluded according to the study performed to OECD guideline 316, that PHMB could be considered as non-photodegradable.

In relation to biotic degradation, a screening test was performed according to OECD guideline 301B, using two concentrations of ^{14}C-PHMB (0.1 and 1.0 mg/l) and $^{14}CO_2$ as mineralization. After 99 days only 3.8% of PHMB was mineralised to CO_2, which demonstrates that the substance is not readily biodegradable. A simulation study conducted according to OECD guideline 303A, imitating the conditions of a sewage treatment plant, showed that PHMB is very slightly mineralized to CO_2. Besides, even with selected and adapted strains, in laboratory conditions and enriched medium, biodegradation of PHMB reached only 29% after 35 days, confirming that the substance is not inherently 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.

The low log Kow (- 2.3) and the high molecular weight (>700g/mol), indicate the absence of potential for PHMB to bioaccumulate.

Aquatic toxicity tests were performed according to EPA standards or OECD guidelines on 3 trophic levels (fish, aquatic invertebrates and algae). All LC50 or EC50 values were below 1 mg/l. The 72h EC50 for algae (the most sensitive species) was equal to 0.015 mg/l.

In conclusion, RAC agrees that the results of the aquatic acute toxicity tests and the non-readily biodegradability justify to classify PHMB as Aquatic Acute 1-H400 and Aquatic Chronic 1-H410 (CLP Regulation) and as N, R50/53 (DSD). Furthermore, in view of the proposed classification and the toxicity band between 0.01 and 0.1 mg/l, RAC proposes an M-factor of 10.

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) 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) with an M-factor of 10 for acute toxicity.

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

Additional information

The Background Document, attached as Annex 1, gives the detailed scientific grounds for the Opinion.

References
See Annex 1

ANNEXES:

Annex 1	Background Document (BD)[1]
Annex 2	Comments received on the CLH report, response to comments provided by the dossier submitter and rapporteurs' comments (excl. confidential information)

[1] The Background Document (BD) supporting the opinion contains scientific justifications for the CLH proposal. The BD is based on the CLH report prepared by a dossier submitter. The original CLH report may need to be changed as a result of the comments and contributions received during the public consultation(s) and the comments by and discussions in the Committees.