Committee for Risk Assessment

RAC

Opinion
proposing harmonised classification and labelling at EU level of

2,2-bis(bromomethyl)propane-1,3-diol

EC Number: 221-967-7
CAS Number: 3296-90-0

CLH-O-0000001412-86-212/F

Adopted

8 June 2018

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8 June 2018

CLH-O-0000001412-86-212/F

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

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

Chemical name: 2,2-bis(bromomethyl)propane-1,3-diol

EC Number: 221-967-7

CAS Number: 3296-90-0

The proposal was submitted by Norway and received by RAC on 18 April 2017.

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Norway 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/harmonised-classification-and-labelling-consultation/* on 23 May 2017. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by 7 July 2017.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: Agnes Schulte

Co-Rapporteur, appointed by RAC: Daniel Borg

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on 8 June 2018 by consensus.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	(603-RST-VW-Y)	2,2-bis(bromomethyl)propane-1,3-diol	221-967-7	3296-90-0	Muta. 1B Carc. 1B	H340 H350	GHS08 Dgr	H340 H350			
RAC opinion	(603-RST-VW-Y)	2,2-bis(bromomethyl)propane-1,3-diol	221-967-7	3296-90-0	Muta. 1B Carc. 1B	H340 H350	GHS08 Dgr	H340 H350			
Resulting Annex VI entry if agreed by COM	(603-RST-VW-Y)	2,2-bis(bromomethyl)propane-1,3-diol	221-967-7	3296-90-0	Muta. 1B Carc. 1B	H340 H350	GHS08 Dgr	H340 H350			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The Dossier Submitter (DS) proposed the classification of 2,2-bis(bromomethyl)propane-1,3-diol (BMP), a flame retardant used in plastics and as an intermediate for production of other chemicals, for mutagenicity (Cat. 1B) and carcinogenicity (Cat. 1B).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS came to the conclusion that a proposal to classify BMP as Muta. 1B is justified on the basis of the available *in vitro/in vivo* genotoxicity data and indications that BMP reaches the germ cells.

In vitro tests

The majority of the available *in vitro* genotoxicity tests conducted with BMP were positive, including three bacterial gene mutation tests (Unknown author, 1996a,b; Zeiger *et al.*, 1992), one chromosomal aberration test (Galloway *et al.*, 1987) and two comet assays (Kong *et al.*, 2011; Kong *et al.*, 2013).

One bacterial gene mutation test was negative (Mortelsmans *et al.*, 1986) and one sister chromatid exchange assay resulted in an equivocal outcome (Galloway *et al.*, 1987).

In vivo tests (somatic cells)

A micronucleus test in peripheral blood cells was positive in male and female mice after feeding with BMP (NTP, 1996). Further micronucleus tests in bone marrow cells of mice showed an inconclusive result in males after oral gavage of BMP, whereas a positive result was observed in males and females after intraperitoneal (i.p.) injection of BMP (NTP, 1996).

An *in vivo* comet assay was positive in urinary bladder cells and negative in liver cells of rats given BMP by gavage (Wada *et al.*, 2014).

In vivo tests (germ cells)

No validated germ cell mutagenicity tests are available.

Treinen *et al.* (1989) showed that BMP exposure leads to reduced fertility, as a specific effect on female reproductive capacity of mice, whereas Bolton *et al.* (1997) observed a reduction in follicle numbers of female mice. The DS concluded that these findings indicate that BMP reaches the germ cells.

Conclusion

On the basis of an analysis and assessment of the available genotoxicity studies with BMP the DS concluded that BMP induces mutagenic effects *in vitro* (in bacteria and in mammalian cell cultures) as well as *in vivo* (in somatic cells of mammals). Additional data from reproductive

reports led to the conclusion that BMP reaches the germ cells. Due to the *in vivo* mutagenicity in somatic cells and the information on systemic availability of BMP, there was some evidence that the substance also has potential to cause mutations in germ cells. Therefore, the DS considered that classification as Muta. 1B (H350) is warranted.

Comments received during public consultation

Two MSCAs agreed with the proposal for classification of BMP as Muta. 1B, while another two MSCAs were of the opinion that classification as Muta. 2 would more be appropriate. For one MSCA, mutagenicity in somatic cells was adequately shown but they considered that there were doubts concerning whether classification as Muta. 1B is appropriate.

Assessment and comparison with the classification criteria

In vitro/in vivo tests with somatic cells

The evaluation of the available genotoxicity data on BMP by the DS and RAC resulted in the same conclusion.

Based on consideration of reliable genotoxicity data, RAC also concludes that the substance induces mutagenic effects *in vitro* as well as *in vivo* in somatic cells.

In vivo tests with germ cells

No germ cell mutagenicity studies with BMP are available.

Other data indicating that the germ cells may be reached

Toxicokinetic data

The only available toxicokinetic data on BMP concentrations in the gonads was given in the publication of Hoehle *et al.* (2009). In the testis of rats only 0.01% BMP was recovered after up to 10 days of exposure. No female rats were used in study.

There were no other toxicokinetic data examining whether BMP reaches the ovaries of mammals.

No data were available on the distribution of the metabolite(s) before and after interal reabsorption, or whether BMP or its glucuronide metabolite is the active compound.

Indications from other studies

Reproductive studies in mice by Treinen *et al.* (1989) and Bolon *et al.* (1997) identified the ovary as a target organ following BMP exposure.

Bolon *et al.* (1997) characterised altered follicle counts as quantifiable endpoints of ovarian injury. BMP, one of the substances studied, significantly decreased the differential counts of follicles in CD-1 mice. The reductions in follicle numbers were proportional to tested doses.

Treinen *et al.* (1989) demonstrated that BMP impaired fertility in female CD-1 mice in a continuous breeding study (in which treatment started 7 days prior to and during a 98 cohabitation period, and the neonates were removed to encourage the next pregnancy (Task 2)), in crossover mating experiments (Task 3) and in the second generation (Task 4).

The significantly reduced numbers of litters per pair during the whole treatment period of the continuous breeding experiment (Task 2) could indicate increased cycle lengths (but there were no data on cycle duration for this group). The authors stated that effects on oestrous cyclicity were not seen in treated females in the crossover experiment (Task 3) and in the second

generation (Task 4). The absence of consistent effects was interpreted that no clear conclusion on the effect on oestrous cyclicity can be drawn.

BMP exposure significantly decreased the numbers of litters per pair, pups born alive per litter, and pup weight when adjusted for litter size in this study. Sperm concentration, motility and morphology in male CD-1 mice were unaffected by BMP exposure. These results suggested the conclusion that BMP (or its active metabolite(s)) could reach the ovaries of female CD-1 mice. It may not have reached (or had only reached in insufficient amounts) or affected the testes of male CD-1 mice.

The number of live pups per litter were significantly lower in the continuous breeding experiment (Task 2), the crossover experiment (Task 3) and the second generation experiment (Task 4) in the Treinen study (Treinen *et al.*, 1989). The lower number of live pups may be interpreted as being consistent with the reduced follicle counts published in the study of Bolon *et al.* (1997), which is assumed to have documented the results of the same test animals (CD-1 mice) from the same NTP laboratory. The dose-related decrease in differential follicle counts in treated mice, the markedly reduced follicle numbers at the high dose (0.4%, i.e. 589 mg/kg bw/d) and the consistency between the results of the two studies gives a strong indication that this is a treatment-related effect.

However, the lower body weight gains of dams in comparison to the control values seen in Tasks 2, 3, and 4 may raise the question whether the effects on the follicles could be secondary to lower body weight. At the highest dose (0.4%, i.e. 589 mg/kg bw/d) the final body weights after continuous breeding (Task 2) were 11-15% lower than the control values, 17% lower in the crossover mating group (Task 3), and 18% lower in the second generation (Task 4). As the numbers of live pups per litters were reduced in all experiments, the reduced body weight gain is likely to have contributed to the smaller litters. Some uncertainty remains as no data on corrected body weights in dams were provided.

One MSCA in their comment suggested that the decrease in follicle counts could be secondary to other effects such as a disruption of the hypothalamic-pituitary axis. The available data from the combined chronic toxicity/carcinogenicity studies in rats and mice do not support this assumption. Although such effects can not be excluded, no indication of effects on the hypothalamic-pituitary axis were observed from the 13 week studies in rats and mice or from the organs at sites where tumours were seen (NTP, 1996).

Based on these data, two conclusions can be drawn:

1. Although it cannot be ruled out with certainty that BMP has the potential to induce mutagenic effects in germ cells, there is no direct evidence of this due to the lack of specific studies on germ cell mutagenicity. There is also no direct evidence that the observed reproductive effects were due to mutations in germ cells. If no other data were available, classification as Muta. 2 would be appropriate (CLP Guidance, 3.5.2.2).

2. The DS, however, in their rationale referred to the following relevant criterion for classification as Muta. 1B in the CLP Regulation (Annex I, Table 3.5.1): "… *positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells*". Data from Bolon *et al.* (1997) and Treinen *et al.* (1989) indicated that BMP reaches the germ cells in female mice.

RAC considered the following arguments:

1. RAC discussed the proposal under the premise given in the CLP Guidance (3.5.2.4): "*It could be argued that in a case where in vivo mutagenicity/genotoxicity is proven and the substance under consideration is systemically available, than that substance should also be considered as a Category 1B mutagen*". According to RAC's interpretation, the criteria

define that evidence for reaching the germ cells in combination with evidence for *in-vivo* mutagenicity may be sufficient to conclude that a substance is a germ cell mutagen.

2. RAC considered the arguments as to whether the data on systemic availability and whether the germ cells were reached were sufficiently robust. It was acknowledged that evidence mainly comes from two sources: (a) the observation of tumours at multiple sites, which was reflected in RAC's proposal to classify BMP as carcinogen Cat. 1B and (b) the effects on the ovarian cells which, although not sufficient for showing that in germ cells mutagenic effects as such have occurred, but demonstrated that the germ cells were reached. The evidence that toxicokinetic studies found a low percentage of the radioactively labelled substance in the testis was considered as supporting evidence that the germ cells were reached. This finding, however, in this case was of lower importance. Unfortunately, no toxicokinetic data were available for female animals.
3. It was noted that BMP impaired fertility, as indicated from the Treinen *et al.* study (1989). A separate documentation of all the data on reproductive toxicity would have been useful, however in the DS proposal a classification proposal only considered selected endpoints. Dams showed lower body weight at the time of parturition (Task 3 and Task 4), for which the lower numbers of live pups/litter cannot be excluded as a cause (as no corrected body weight data were available). Body weight effects linked to lower pup numbers made maternal toxicity appear less likely as being responsible for the effect on follicles. Based on the available data, there is no indication of a hormone-related effect.
4. It might appear inconsistent that no ovary effects were observed in other repeated dose toxicity studies (e.g. from the NTP report). However, these studies did not include morphometric analyses of small and growing follicles and the absence or presence of effects on follicle development remains uncertain. Finally, the reduced numbers of small and growing follicles were reproduced in two sets of studies in the Bolon *et al.* publication (1997) and showed dose-dependency. No arguments were found to disregard the evidence (from the cancer studies and from studies of Treinen/Bolon) that female germ cells were reached and that the substance interacted with these cells.

In conclusion, RAC agreed with the DS's proposal that there is positive evidence of somatic cell mutagenicity from *in vitro/in vivo* studies and evidence from the reproductive toxicity studies support that BMP reaches the (female) germ cells. Both facts in combination are sufficient to give 'some' evidence that the substance has the potential to cause mutations to germ cells.

Therefore, RAC agrees with the DS' conclusion that BMP should be classified as a germ cell mutagen, Cat. 1B; H340.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS summarised two carcinogenicity studies; one study in rats and mice performed by the National Toxicology Program (NTP) and one study in rats performed by industry. Two supportive studies were also included, one mechanistic follow-up study to the NTP-study and one 90-day repeated dose toxicity study in rats and mice.

2-year rat/mouse study (Dunnick et al., 1997; NTP, 1996)

In the carcinogenicity study by the NTP, conducted under GLP and in conformity with OECD TG 453, F-344 rats and B6C3F1 mice were dosed with the technical mixture FR-1138 orally through feed. The FR-1138 contained 78.6% 2,2-bis(bromomethyl)propane-1,3-diol (BMP); 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane, 6.9% 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane, 0.2% pentaerythritol and 7.7% structural isomers and dimers.

Rats

F-344 rats (60/sex/dose) were provided FR-1138 in the diet at 0, 2500, 5000 or 10000 ppm (males: 0, 100, 200, 430 mg/kg bw/day; females: 0, 115, 230, 460 mg/kg bw/day) for 2 years. A stop-exposure group was also included where 60 male rats received 20000 ppm FR-1138 (800 mg/kg bw/day) for 3 months and then fed control diet for the remainder of the study.

General toxicity consisted of decreased body weight gain and terminal body weight in the exposed groups as compared to the controls, resulting in mean terminal body weights up to 14% lower in males in the two high dose groups and up to 5% lower in females in the high dose group. Food consumption was generally similar to that of controls. Decreased survival occurred in the mid and high dose males and females primarily due to treatment-related neoplasms. Clinical findings consisted of cutaneous and subcutaneous tissue masses on the face, tail and on the dorsal and ventral surfaces of exposed rats.

The DS reported treatment-related neoplastic and non-neoplastic lesions combined for 17 tissues in male rats (skin, subcutaneous tissue, Zymbal gland, oral cavity, oesophagus, forestomach, small intestine, large intestine, peritoneum, kidney, urinary bladder, lung, thyroid, seminal vesicle, haematopoietic system, mammary gland and pancreas) and five tissues in female rats (mammary gland, oral cavity, oesophagus, kidneys and thyroid). The data indicated dose-response relationships for several of these tumours. The effects observed in the kidney and pancreas of male rats were predominantly hyperplastic, thus the DS considered the effects in these tissues of male and female rats as equivocal with regard to carcinogenicity and reported *clear or some evidence* of treatment-related carcinogenic effects in 15 tissues in male rats and four tissues in female rats (Table below).

Mice

B6C3F1 mice (60/sex/dose) were given FR-1138 in the diet at 0, 312, 625, 1250 ppm (males 0, 35, 70, 140 mg/kg bw/day; females 0, 40, 80, 170 mg/kg bw/day) for 2 years.

Decreased survival was reported at the highest dose in males and females, primarily due to treatment-related neoplasms. Mean body weights of exposed male and female mice as well as food consumption were similar to controls throughout the study. Clinical findings in exposed mice consisted of swelling, discharges and tissue masses in the eyes (related to the tumours of the Harderian gland).

The DS reported treatment-related neoplastic and non-neoplastic lesions combined for four tissues in males (Harderian gland, lung, forestomach and kidney) and 6 tissues in females (Harderian gland, lung, subcutaneous tissue, forestomach, mammary gland and circulatory system) and the data indicated a dose-response relationships for several tumour types. The DS reported *clear or some evidence* of treatment-related carcinogenic effects in four of the tissues in male mice and three of the tissues in female mice (Table below).

Table: Summary table of carcinogenic effects in 2-year studies of BMP in rats and mice (Dunnick et al., 1997; NTP, 1996).

Site	Rats		Mice	
	Male	Female	Male	Female
Skin	+			
Subcutaneous tissue	+			+
Mammary gland	+	+		+/-
Zymbal's gland	+			
Oral cavity	+	+		
Oesophagus	+	+		
Forestomach	+		+	+/-
Small intestine	+			
Large intestine	+			
Mesothelium	+			
Kidney	+/-		+	
Urinary bladder	+			
Lung	+		+	+
Thyroid gland	+	+		
Seminal vesicle	+			
Haematopoietic system	+			
Pancreas	+/-			
Harderian gland			+	+
Circulatory system				+/-

+ = some or clear evidence of carcinogenic activity; +/- = equivocal evidence of carcinogenic activity

The DS considered the outcome of this NTP-study as evidence of carcinogenicity of BMP in rodents. In the technical mixture FR-1138, containing ~80% BMP, the two main impurities were monobromoneopentyl triol (6.6%) and tribromoneopentyl triol (6.9%). The DS referred to ECHA screening, for substances likely to fulfil the criteria for inclusion in REACH Annex III, which showed indications for concern for monobromoneopentyl triol as a suspected mutagen and carcinogen. Studies for BMP and tribromoneopentyl triol from the NTP-program showed almost identical genotoxicity properties. Altogether, the DS considers these major impurities to have similar properties to BMP.

2-year rat study (Keyes et al., 1980)

Sprague-Dawley rats (49-50/sex/dose) were given FR-1138 in the diet at 0, 5 or 100 mg/kg bw/day for 2 years. The FR-1138 was reported to contain 80% BMP, 8% tribromeopentyl alcohol, 6% monobromeopentyl triol and 3% of other impurities.

No evidence of carcinogenicity was reported in the study. General toxicity was low, with only slight reductions in body weight in treated males. Survival was not significantly different among the groups. The high dose, 100 mg/kg bw/day, is comparable to the low-dose in the NTP-study described above (Dunnick *et al.*, 1997; NTP, 1996).

Mechanistic follow-up study to the NTP carcinogenicity-study (NTP, 1996; Ton et al., 2004)

In a follow-up study to the NTP-carcinogenicity study (Dunnick *et al.*, 1997; NTP, 1996), lungs from the B6C3F1 male and female mice were fixed, DNA isolated and characterised for mutations in the protooncogene K-ras. The result showed that the frequency of mutated K-ras in BMP-induced lung neoplasms (57%) was significantly elevated over that of spontaneous lung

neoplasms from control mice (15%). The DS considered this study to support the observed lung tumours and that this may indicate direct or indirect genotoxicity of BMP or its metabolites.

90-day study in rats/mice (Elwell et al., 1989; NTP, 1996)

In a 90-day dose-range finding study to the 2-year carcinogenicity study, F-344/N rats were dosed with FR-1138 either via gavage at 0, 50, 100, 200, 400 and 800 mg/kg bw/day or via the diet at 0, 1250, 2500, 5000, 10000 and 20000 ppm (males: 0, 100, 200, 400, 800, 1700 mg/kg bw/day; females: 0, 100, 200, 400, 800, 1600 mg/kg bw/day). Similarly, B6C3F1 mice were exposed to BMP at 0, 25, 50, 100, 200 and 400 mg/kg bw/day or via the diet at 0, 625, 1250, 2500, 5000 and 10000 ppm (males: 0, 100, 200, 500, 1300, 3000 mg/kg bw/day; females: 0, 140, 300, 600, 1200, 2900 mg/kg bw/day).

The results showed that the kidneys and bladder were target organs and that mice were more sensitive than rats for kidney and bladder lesions. Transitional cell hyperplasia was observed at the higher doses, with potential to be transformed into tumours. The DS considered this study as supportive evidence for the carcinogenic effect of BMP seen in the NTP carcinogenicity study.

Overall, the DS considered that there is sufficient evidence from the NTP-study of FR-1138 (containing ~80% BMP) in two species, rats and mice, showing substance and dose-related inductions of multisite human relevant tumours in both sexes, which warrants classification of BMP as Carc. 1B.

Comments received during public consultation

Five MSCA's commented on the classification proposal. All supported classification of BMP as Carc. 1B. Two MSCA's considered the preneoplastic lesions observed in two studies on rats and mice as supporting evidence. One MSCA did not consider the negative results in the industry study to weaken the strength of evidence for carcinogenicity considering the lower doses used in that study in comparison with the NTP-study.

One MSCA disagreed with the DS's statement "clear exposure-related carcinogenic effects were observed at 17 sites in male rats (skin, subcutaneous tissue, mammary gland, Zymbal gland, oral cavity, oesophagus, forestomach, small intestine, large intestine, mesothelium, kidney, urinary bladder, lung, thyroid gland, seminal vesicle, hematopoietic system, and pancreas)". The DS referred to two of these tissues, which in their view did not indicate dose-related effects, and also suggested inclusion of historical control data (HCD). The DS responded that they have cited the study authors' conclusions regarding "exposure-related carcinogenic effects" and that the HCD is available in the NTP report (NTP, 1996).

Assessment and comparison with the classification criteria

Two carcinogenicity studies were provided on FR-1138, a technical mixture containing ~80% BMP; one study in rats and mice conducted by the National Toxicology Program under GLP and in accordance with OECD TG 453 (Dunnick *et al.*, 1997; NTP, 1996) and one study in rats conducted by industry (Keyes *et al.*, 1980). Two supportive studies were also included.

In the NTP-study, BMP induced treatment-related tumours at multiple sites in rats and mice (relevant Tables below). General toxicity consisted of decreased body weight in the rats, an average reduction of up to 14% in the two male high dose groups relative to controls, and decreased survival in the mid and high dose male and female rats as well as high dose male and female mice, primarily related to the presence of treatment-related neoplasms (preterm sacrifice).

In male rats, dose-related increases of benign and malignant tumours in a number of tissues were observed following exposure to BMP for 2 years - skin, subcutaneous tissue, Zymbal's gland, oral cavity, oesophagus, forestomach, small intestine, large intestine, urinary bladder, lung and seminal vesicles, (Table below). The stop-exposure group showed that BMP dosing for only 3 months induced tumours at most sites where tumours were observed following 2-year exposure, in some tissues to a greater extent. The increase in subcutaneous neoplasms were the primary reason for preterm sacrifice of moribund rats in the high-dose groups. In a number of other tissues, increased but not clearly dose-dependent incidences of tumours were observed - mammary gland, thyroid gland and the haematopoietic system, which were outside the range of the HCD. Increased incidences of adenomas in the kidney and pancreas were observed in the exposed groups as compared to controls, however these were within the range of the HCD.

Table: Incidences (%) of selected treatment-related neoplasms in male F344/N Rats (n=51-60/group) following 2-years exposure to BNP (Dunnett et al., 1997; NTP, 1996).

Site	Dose (mg/kg bw/day)					HCD
	0	100	200	430	800#	
Skin (adenoma, papilloma, carcinoma)	8	11	27**	44**	35**	2-16
Subcutaneous tissue (fibroma, fibrosarcoma, sarcoma)	4	17*	25**	29**	17**	0-16
Mammary gland (adenoma, fibroadenoma)	0	8*	14**	13**	8**	0-12
Zymbal's gland (adenoma, carcinoma)	4	2	8	9	25**	0-4
Oral cavity (papilloma, carcinoma)	0	8*	18**	18**	22**	0-4
Oesophagus (papilloma, carcinoma)	0	0	2	11*	0	N.A.
Forestomach (papilloma)	0	0	0	2	8	0-2
Small intestine (adenoma, carcinoma)	0	0	0	4	8*	0-4
Large intestine (adenoma, carcinoma)	0	0	6	7	17	0-2
Peritoneum (malignant mesothelioma)	0	6	16**	16**	43**	0-8
Kidney (renal tubule adenoma)	0	0	2	5**	2	0-6
Urinary bladder (papilloma, carcinoma)	0	0	2	5	3	0-2
Lung (adenoma, carcinoma)	2	2	6	7*	12*	0-8
Thyroid gland (adenoma, carcinoma)	0	4	12*	5	15**	0-6
Seminal vesicle (adenoma, carcinoma)	0	0	0	0	3	0
Haematopoietic system (mononuclear cell leukaemia)	53	55	78**	62**	42**	32-62
Pancreas (adenoma)	2	4	8*	6	5	0-10

**p<0.05 vs controls, **p<0.01 vs controls, N.A. = not available, #Dosed for 3 months and then maintained on a control diet.*

In female rats, dose-related increases in benign and malignant tumours in the oral cavity, oesophagus and thyroid gland were observed (Table below). In the mammary gland, increased, though not clearly dose-related, incidences of benign and malignant tumours were observed, which were outside the range of the HCD.

Table: Incidences (%) of selected treatment-related neoplasms in female F344/N Rats (n=50-52/group) following 2-years exposure to BNP (Dunnett et al., 1997; NTP, 1996)

Site	Dose (mg/kg bw/day)				HCD
	0	115	230	460	
Oral cavity (papilloma, carcinoma)	4	6	9	12	0-6
Oesophagus (papilloma)	0	0	2	19**	N.A.
Mammary gland (fibroadenoma, adenoma, carcinoma)	54	92**	89**	90**	8-64
Thyroid gland (adenoma, carcinoma)	0	0	4	8**	0-6

***p<0.01 vs controls, N.A. = not available*

In male mice, dose-related increases of benign and malignant tumours in the Harderian gland, lung and forestomach were observed (Table below). Increased incidences of adenomas in the kidney outside the HCD were observed, although these were not clearly dose-related.

Table: Incidences (%) of selected treatment-related neoplasms in male B6C3F1 mice (n=49-51/group) following 2-years exposure to BNP (Dunnett et al., 1997; NTP, 1996)

Site	Dose (mg/kg bw/day)				HCD
	0	35	70	140	
Harderian gland (adenoma, carcinoma)	8	14	32**	45**	0-20
Lung (adenoma, carcinoma)	30	22	32	51*	4-32
Forestomach (papilloma, carcinoma)	0	6	6	8*	0-6
Kidney (adenoma)	0	0	3	2	0-2

**p<0.05 vs controls, **p<0.01 vs controls*

In female mice, dose-related increases of benign and malignant tumours in the Harderian gland, lung, subcutaneous tissue and mammary gland were observed (Table below). Increased incidences of tumours in the mammary gland and circulatory system were observed compared to the controls, however these were within the range of the HCD.

Table: Incidences (%) of selected treatment-related neoplasms in female B6C3F1 mice (n=50-52/group) following 2-years exposure to BNP (Dunnett et al., 1997; NTP, 1996)

Site	Dose (mg/kg bw/day)				HCD
	0	40	80	170	
Harderian gland (adenoma, carcinoma)	6	24**	25**	38**	0-10
Lung (adenoma, carcinoma)	10	10	29**	38**	2-26
Subcutaneous tissue (fibrosarcoma, sarcoma)	0	2	8	24**	0-8
Forestomach (papilloma)	0	2	10*	6*	0-14
Mammary gland (carcinoma)	0	0	2	6	0-10
Circulatory system (haemangioma, haemangiosarcoma)	2	4	0	10*	0-8

**p<0.05 vs controls, **p<0.01 vs controls*

Altogether, the outcome of this carcinogenicity study showed that exposure to FR-1138 caused multi-site tumours in both sexes of rats and mice without any overt signs of general toxicity, warranting classification as Carc. 1B. The stop-exposure group in male rats showed that BMP administered for only 3 months was carcinogenic and that the incidences of tumours in some tissues were greater than in the continuous-exposure groups.

In a follow-up study (Ton *et al.*, 2004) lungs from the B6C3F1 mice were characterized for mutations in the protooncogene K-ras. The frequency of mutated K-ras in BMP-induced lung neoplasms was significantly elevated over that of spontaneous lung neoplasms from control mice (57% vs 15%) and indicated that mutations in the K-ras gene could be involved in the observed lung carcinogenesis. However, RAC considered the result of this single study as providing limited mechanistic support for a genotoxic mechanism of action of BMP.

In the other 2-year carcinogenicity study in SD-rats performed by industry (Keyes *et al.*, 1980), no statistically significant treatment-related increases of tumour incidences were reported.

However, only two doses were used, 5 and 100 mg/kg bw/day, which were considered low in comparison to the NTP study, with the high dose 100 mg/kg bw/day being equal to the low dose in the NTP study. In this study, the general toxicity was low, with only slight reductions in body weight in treated males and survival not significantly different among the groups. Thus, this study was given limited weight in the assessment.

The 90-day study (Elwell *et al.*, 1989) showed that kidney and bladder in both rats and mice are target organs for BMP, with renal papillary necrosis and renal tubular regeneration and fibrosis observed as well as urinary bladder hyperplasia. This study provided support to the effects seen in the NTP carcinogenicity study.

RAC conclusions

BMP induced dose-dependent multi-site tumours in two species, rats and mice, in a well-conducted OECD TG 453-study carried out by the NTP under GLP conditions and with limited general toxicity. Both benign and malignant tumours were observed in the respective tissues, showing the ability of the tumours to progress to malignancy. The stop-exposure group in male rats showed that only 3 months of exposure induced tumours at most sites where tumours were observed in the 2-year continuous-exposure groups. The incidences of neoplasms were greater at some sites (lungs, small and large intestine, thyroid). Adenoma and carcinoma of the seminal vesicle were also found, which did not occur in the other groups, and which are extremely rare in rats. Based on the findings from this group, genetic damage appears to occur within the first few months of exposure and that can develop into tumours, also in the absence of a toxic response in these tissues. Some of the tumours observed fit into the pattern of genotoxic chemicals (NTP, 1996).

One negative study in rats was included in the evaluation, however the study was conducted at doses similar to and lower than in the NTP-study and is therefore given less weight. Some support has also been provided on preneoplastic lesions in some target organs of a 90-day study, also indicating lack of preneoplastic lesions in many target tissues, and indications of genotoxicity were presented in a follow-up study on tissue material from the NTP-study.

Altogether, RAC consider BMP to be a multi-site carcinogen in two species with tumours of human relevance. Therefore, RAC agrees with the DS's proposal to classify BMP as Carc. 1B; H350.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).