Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

barium diboron tetraoxide

EC Number: 237-222-4
CAS Number: 13701-59-2

CLH-O-0000006847-60-01/F

Adopted

17 September 2020

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CLH-O-0000006847-60-01/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: **barium diboron tetraoxide**

EC Number: **237-222-4**

CAS Number: **13701-59-2**

The proposal was submitted by **Sweden** and received by RAC on **9 August 2019.**

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

PROCESS FOR ADOPTION OF THE OPINION

Sweden 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 **14 October 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **13 December 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Michal Martínek**

Co-Rapporteur, appointed by RAC: **Annemarie Losert**

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 **17 September 2020** by **consensus**.

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

	Index No	Chemical name	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	056-002-00-7	barium salts, with the exception of barium sulphate, salts of 1-azo-2-hydroxynaphthalenyl aryl sulphonic acid, and of salts specified elsewhere in Annex VI of 1272/2008			Acute Tox. 4* Acute Tox. 4*	H332 H302	GHS07 Wng			*	A1
Dossier submitter's proposal	TBD	barium diboron tetraoxide	237-222-4	13701-59-2	**Add** Repr. 1B **Modify** Acute Tox. 4 **Remove** Acute Tox. 4*	**Add** H360FD **Retain** H302 **Remove** H332	**Add** GHS08 **Retain** GHS07 **Modify** Dgr	**Add** H360FD **Retain** H302 **Remove** H332		**Add** oral: ATE = 530 mg/kg bw	
RAC opinion	TBD	barium diboron tetraoxide	237-222-4	13701-59-2	**Add** Repr. 1B **Modify** Acute Tox. 4 Acute Tox. 3	**Add** H360FD **Modify** H301 **Retain** H332	**Add** GHS08 **Modify** GHS06 Dgr	**Add** H360FD **Modify** H301 **Retain** H332		**Add** inhalation: ATE = 1,5 mg/L (dusts or mists) oral: ATE = 100 mg/kg bw	
Resulting Annex VI entry if agreed by COM	TBD	barium diboron tetraoxide	237-222-4	13701-59-2	Repr. 1B Acute Tox. 4 Acute Tox. 3	H360FD H332 H301	GHS08 GHS06 Dgr	H360FD H332 H301		inhalation: ATE = 1,5 mg/L (dusts or mists) oral: ATE = 100 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Barium diboron tetraoxide is currently covered by a group entry with a harmonised classification for acute toxicity. The dossier submitter (DS) proposed a separate entry for barium diboron tetraoxide (hereafter barium metaborate) with a harmonised classification for reproductive toxicity in addition to acute oral toxicity. The assessment of reproductive toxicity is based on data on the substance itself (toxicity data generated with Busan 11-M1, a commercial form of barium metaborate monohydrate) and on read-across from boric acid and borax (disodium tetraborate decahydrate).

Previous RAC evaluations of boric acid and borates

Harmonised classification of boric acid and several related compounds with Repr. 1B; H360FD was added into Annex VI of the CLP regulation in its 1st ATP (Commission Regulation (EC) No 790/2009). In 2014, RAC evaluated a proposal to downgrade the classification of boric acid to Repr. 2; H361d, but concluded that Repr. 1B; H360FD should be retained. RAC also evaluated reproductive toxicity of disodium octaborate at that time and agreed on Repr. 1B; H360FD, based on read-across from boric acid and borax.

In 2019, RAC agreed that specific concentration limits (SCL) for boric acid (SCL 5.5%) and several other boron compounds classified as Repr. 1B should be removed and the generic concentration limit of 0.3% should apply.

Read-across from boric acid and borax to barium metaborate

The available information on reproductive toxicity of barium metaborate is limited to a 90-day study in rats and a prenatal developmental toxicity (PNDT) study in rabbits. To complete the information, the DS proposed read-across from boric acid and borax. Studies with barium chloride have been presented to provide information on the toxicity of the barium cation.

The guidance document 'Read-Across Assessment Framework' (ECHA, 2017) lists several key elements to be assessed in cases where the read-across is based on formation of common compound(s):

1. Formation of common compounds
2. The biological targets for the common compounds
3. Exposure of the biological targets for the common compounds
4. The impact of parent compounds
5. Formation and impact of non-common compounds

<u>Formation of a common compound</u>

Metal borates generally dissolve in water to form boric acid, $B(OH)_3$, and tetrahydroxyborate anion, $[B(OH)_4]^-$. As the pK_a for the reaction

$$B(OH)_3 + 2\ H_2O \leftrightarrows [B(OH)_4]^- + H_3O^+$$

is approx. 9, boric acid is the predominant species in acidic environments (pH < 5). At higher concentrations and intermediate pH values where both $B(OH)_3$ and $[B(OH)_4]^-$ are present in the solution, polynuclear complexes composed of BO_3 and BO_4 units are also formed. In addition, metal cations form metal-ion complexes with the tetrahydroxyborate anion. Overall, boric acid is

the main species at the pH values in the gastrointestinal tract. Thus, barium metaborate, $Ba(BO_2)_2$, is expected to convert to boric acid and barium cations in the stomach. Borax, $Na_2[B_4O_5(OH)_4]\cdot 8H_2O$, converts to boric acid and sodium cations.

Publicly available information indicates that the commercial form of barium metaborate tested in the toxicology studies (Busan 11-M1) is treated with an additive in order to reduce its water solubility. This may theoretically lead to slower dissolution and absorption and consequently a quantitative difference in the toxicological profile when compared to boric acid or borax. However, the effect levels for impaired spermatogenesis in the 90-day rat dietary study with Busan 11-M1 (marked effect at 64 mg B/kg bw/d; Study report, 1993a) are similar to those in the rat dietary studies with boric acid and borax (Weir and Fisher, 1972: marked effect at 47 mg B/kg bw/d; Ku *et al.*, 1993: marked effect from 38 mg B/kg bw/d), when compared on the basis of boron equivalents. Likewise, the rat oral LD_{50} for Busan 11-M1 (850/530 mg/kg bw in males and females, respectively) is only slightly higher than that for barium chloride (ca. 200 to 650 mg/kg bw; barium content is similar). Thus, the coating does not appear to markedly affect absorption from the gastrointestinal tract.

As for instance stated in the IPCS report on boron (WHO, 1998), the chemical and toxicological effects of boric acid and other borates are similar on a mol boron/L equivalent basis when dissolved in water or biological fluids at the same pH and low concentration. Therefore, comparison on the basis of boron equivalents is justified.

The following conversion factors are based on the molar masses of barium diboron tetraoxide, boric acid and boron and can be used to calculate boron equivalents for the different substances:

Equivalent boron weight = weight of barium diboron tetraoxide x 0.0897
Equivalent boron weight = weight of boric acid x 0.1750
Equivalent boron weight = weight of disodium tetraborate decahydrate (borax) x 0.1133

Biological targets for the common compound

As the transformation of barium metaborate or borax to boric acid occurs already in the stomach, *i.e.* before absorption, the biological targets of all three substances are expected to be the same.

Exposure of the biological targets for the common compound

Comparison of toxicity data for the source substances and the target substance (see above) does not indicate a significant quantitative difference in exposure of testes to the common compound (boric acid). The same is assumed for other biological targets.

The impact of parent compounds

The substance is assumed to hydrolyse to boric acid upon dissolution. Since undissolved barium metaborate is a solid with a polymeric or oligomeric structure, not available for absorption, exposure of the biological targets to the parent compound is not expected.

Formation and impact of non-common compounds

Boric acid is absorbed unchanged. Borax is transformed to boric acid with a concomitant release of sodium cations; the amount of sodium released from borax is not assumed to contribute to the toxicity of borax at the dose levels tested.

Barium shows higher general toxicity than the borate. Information on the toxicity of the barium cation in animals is available from studies with barium chloride, $BaCl_2$, a soluble barium salt. The most prominent features of barium toxicity in rodents are lethality and renal toxicity. No effects on reproduction or on reproductive organs were observed in a PNDT study in rats (Study report, 2014), in repeat dose studies in rats and mice (NTP, 1994) or in a non-guideline reproductive

screening in rats and mice (Dietz *et al.*, 1992). RAC notes that no standard generational study and no rabbit PNDT study are available for barium chloride.

Regarding read-across, it is likely that some of the reproductive effects at higher doses of boric acid or borax would be accompanied by marked general toxicity due to barium, should a similar study be conducted with barium metaborate. This has to be taken into account when evaluating the contribution of the individual studies with boric acid or borax to the classification of barium metaborate.

Toxicity thresholds of $BaCl_2$ are known only for rats and mice and administration routes via gavage or drinking water. No dietary studies in rats or mice are available, nor is data on toxicity thresholds of $BaCl_2$ in dogs and rabbits.

Barium chloride has almost the same barium content (66%) as barium metaborate (62%), so the dose levels can be compared directly. In a 10-day gavage study non-pregnant rats tolerated ca. 200 mg/kg bw/d $BaCl_2$ without symptoms but several animals died at 300 mg/kg bw/d (Borzelleca *et al.*, 1988). Pregnant rats might be more sensitive: 175 mg/kg bw via gavage caused mortality after a single dose and 100 mg/kg bw/d after multiple doses in a recent PNDT study (Study report, 2014). However, there may be other factors besides pregnancy status behind this difference (e.g. different strain, CD in Borzelleca *et al.* 1988 vs. Wistar in Study report, 2014) and therefore the evidence for higher sensitivity of pregnant rats to general toxicity of barium is not considered very robust.

In a subchronic study where $BaCl_2$ was administered via drinking water, rats tolerated approx. 170 mg/kg bw/d while 300 mg/kg bw/d caused mortality. High mortality was observed in a 90-day mouse study at ca. 700 mg/kg bw/d (also via drinking water) while 300 mg/kg bw/d did not cause toxic effects (NTP, 1994).

Conclusion on read-across

The available information indicates that barium metaborate is converted relatively rapidly to boric acid and barium cation in the stomach. Read-across from boric acid and borax to barium metaborate is considered acceptable.

The general toxicity of the barium cation has to be taken into account when evaluating the contribution of the individual studies with boric acid or borax to the classification of barium metaborate.

RAC notes the lack of a standard generational study and a PNDT study in rabbits with a soluble barium salt.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The current harmonised classification for the group entry covering barium metaborate is Acute Tox. 4* for both the oral and inhalation route.

Acute toxicity studies with barium metaborate monohydrate (Busan 11-M1) are available for all three routes: an acute oral toxicity study in rats, an acute dermal toxicity study in rabbits and an acute inhalation toxicity study in rats. Based on the results of these studies the DS proposed Acute Tox. 4 with and ATE of 530 mg/kg bw for the oral route and no classification for the dermal and inhalation route.

The DS also presented information on acute toxicity of boric acid, sodium borates and barium chloride. As there was conclusive information on barium metaborate and the additional information on the related compounds did not contradict the classification derived from data on barium metaborate itself, read-across for acute toxicity was not applied.

Comments received during the consultation

One MSCA commented and supported the DS's proposal.

Assessment and comparison with the classification criteria

Acute oral toxicity

In a pre-guideline acute oral toxicity study (Study report, 1979a) with a design comparable to OECD TG 401, male and female rats were administered barium metaborate monohydrate (Busan 11-M1) in an unspecified vehicle. The LD_{50} was 850 mg/kg bw and 530 mg/kg bw for males and females respectively.

In a 5-day tolerability study in non-pregnant female rabbits (2 animals/group) conducted prior to the main PNDT study with barium metaborate, no mortality was observed at 100 mg/kg bw/d. At the next higher dose of 200 mg/kg bw/d both animals died or had to be sacrificed after a single exposure. In the subsequent range-finding PNDT study (i.e. a study with pregnant females), mortality after a single dose started from 125 mg/kg bw/d, and 4 out of 7 animals died after a single dose of 160 mg/kg bw. When taking into account deaths within the first 72 hours (cf. Guidance on the application of the CLP criteria; CLP guidance, version 5.0, 3.1.1), 50% mortality was reached at 90 mg/kg bw/d in the range-finding PNDT study. Mortality in these studies can be attributed to the barium cation since no mortalities were observed at a considerably higher equivalent dose in a PNDT study with boric acid via gavage in the same strain (Price *et al.*, 1996b).

RAC notes that humans may be generally more sensitive to the acute toxicity of barium compounds than rats. Lethal doses of barium chloride as low as 11 mg/kg bw have been reported in humans (WHO, 2001) while the rat LD_{50} values for this compound range from about 200 to 650 mg/kg bw. Thus, although the rat is the preferred species for acute oral toxicity classification (CLP, Annex I, 3.1.2.2.1), data from the more sensitive species, i.e. rabbit, are preferred in this case, noting that humans might be even more sensitive than rabbits. The rabbit data correspond to Category 3 (50 mg/kg bw < ATE ≤ 300 mg/kg bw).

As to the ATE, no standard LD_{50} can be derived from the rabbit studies due to their design (repeated dosing, no 14-day post-exposure period, low number of animals in the 5-day study), although the LD_{50} appears to lie around 100 mg/kg bw. The converted ATE for Category 3 of 100 mg/kg bw (CLP, Annex I, Table 3.1.2) is considered appropriate.

In order to properly take into account human data, a comprehensive literature search for quantitative information on acute toxicity of barium to humans would have to be performed, which is beyond the RAC mandate. However, RAC recommends that acute toxicity classifications of barium compounds be reviewed in the future in order to ascertain if human data warrant a more stringent classification.

In conclusion, RAC proposes to classify barium metaborate as **Acute Tox. 3; H301** with an **ATE** of **100 mg/kg bw** based on mortality in non-pregnant and pregnant female rabbits.

Acute dermal toxicity

In a pre-guideline acute dermal toxicity study (Study report, 1979b) barium metaborate monohydrate (Busan 11-M1) moistened with physiological saline was applied to abraded skin of

male and female rabbits (5/sex/group) for 24 hours under occlusive conditions at the limit dose of 2000 mg/kg bw. One female died and the LD_{50} was > 2000 mg/kg bw. RAC agrees with the DS's proposal of **no classification**.

Acute inhalation toxicity

In an acute inhalation toxicity (Study report, 1983) study with a design similar to OECD TG 403, male and female rats (5/sex/group) were exposed to barium metaborate monohydrate (Busan 11-M1) as a powder for 4 hours via whole-body exposure. This was followed by a 14 days post exposure observation period. After exposure, all animals in the two treated groups appeared languid from 30 min through 4 hours. One male was found dead at the low concentration of 2.98 mg/L (MMAD 3.4 µm) on day 2 after exposure and 1 female died at the maximum attainable concentration of 3.54 mg/L (MMAD 2.8 µm) on day 1. Some animals of the low concentration group showed slight dyspnoea from hour 2 through and rhinorrhoea from hour 1 through hour 4. On day 1 post exposure individuals of the high concentration group showed clinical signs including lethargy, blood crusts around the nose, polypnoea and wheezing. All remaining animals in this group appeared normal from day 2 through termination. It was concluded that the LC_{50} was > 3.5 mg/L.

The available animal study indicates that the LC_{50} of barium metaborate monohydrate (Busan 11-M1) is greater than the maximum attainable concentration, but two animals died at doses relevant for classification in Category 4.

RAC notes that humans may be generally more sensitive to the acute toxicity of barium compounds than rats. Lethal oral doses of barium chloride as low as 11 mg/kg bw have been reported in humans (WHO, 2001) while the rat LD_{50} values for this compound range from about 200 to 650 mg/kg bw. A single case of severe intoxication after inhalation exposure, where a contribution of oral exposure cannot be excluded (Shankle and Keane, 1988) was located and both, US CDC (2003) and ATSDR (2007), state that barium intoxication was observed after inhalation exposure, but without relevant information on exposure concentration. The available information indicates that inhalation exposure is a relevant route for soluble barium compounds. No in depth analysis of human intoxication cases with soluble barium compounds was included in the CLH report or provided during the consultation. However, RAC considered these data rather relevant for the assessment of acute toxicity of barium metaborate and prepared a crude assessment of the available human data under section "Additional key elements". In order to properly take into account human data, a comprehensive literature search for quantitative information on acute toxicity of barium to humans would have to be performed, which is beyond the RAC mandate. The RAC analysis of the available human data indicates considerable acute toxicity of soluble barium salts via the oral route, and indicates relevance of the inhalation route, supporting the need for a classification.

As no reliable effect level can be derived based on human data and the basis for the original classification of the barium salts entry 056-002-00-7 of Annex VI as Xn; R20 is not known, RAC recommends to keep the classification as Acute Tox. 4; H332. However, RAC recommends that acute toxicity classifications of barium compounds be reviewed in the future in order to ascertain if human data would warrant a more stringent classification.

The current classification of entry 056-002-00-7 for acute inhalation toxicity is marked with an asterisk, indicating that the classification has been translated from the Dangerous Substances Directive (DSD). However, as the borders for classification as Acute Tox. 4, inhalation (dusts and mists) are the same in CLP as for Xn; R20 in the DSD (i.e. 1 – 5 mg/L), RAC is of the opinion that the asterisk can be removed.

A converted ATE of 1.5 mg/L is considered appropriate (CLP, Annex I, Table 3.1.2).

In conclusion, RAC proposes a classification of barium metaborate as **Acute Tox. 4; H332** with an **ATE** of **1.5 mg/L (dusts or mists)**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The DS proposed classification in Category 1B based on severe aspermatogenesis in a 90-day rat study with barium metaborate, further supported by read-across from boric acid and borax, which have been shown to cause alterations to the male reproductive system and impaired fertility in several species. The overall negative human data on boric acid and borates were not considered to contradict the animal data due to methodological limitations of the epidemiology studies and exposure levels below the NOAELs in animals. The DS concluded that an SCL is not warranted since the ED_{10} for testicular atrophy corresponds to the medium potency group.

Development

The only developmental study with barium metaborate available, a PNDT study in the rabbit, was negative. The DS proposed classification in Category 1B based on read-across from boric acid, which caused severe developmental effects such as resorptions and malformations in several species (rat, rabbit, mouse). Human data were not considered to contradict the animal data. The DS concluded that an SCL is not warranted since the LOAEL for short rib XIII in the rat corresponds to the medium potency group.

Lactation

The DS proposed no classification due to absence of robust evidence of adverse effects on or via lactation in the available studies with boric acid and borax. No relevant information was identified on barium metaborate or barium chloride.

Comments received during the consultation

One MSCA supported the DS's proposal, noting that the reproductive toxicity classification of boric acid had already been assessed by RAC and was not contradicted by data on barium metaborate itself.

Assessment and comparison with the classification criteria

Adverse effects on fertility and sexual function

<u>90-day dietary study in rats with barium metaborate (Study report, 1993a)</u>

The study was carried out according to US EPA guidelines and under GLP. The top dose of 10000 ppm (707/794 mg/kg bw/d, equivalent to 64/71 mg B/kg bw/d in males/females) caused body weight reduction (by 10%, males and females), reduced food consumption and decreased haemoglobin (by 7%/9% in males and females, respectively).

RAC notes that doses similar to the relatively well tolerated top dose in this 90-day study were lethal in the acute oral toxicity study (Study report, 1979a); this difference in toxicity can be explained by a different way of administration (diet vs. gavage, with gavage leading to a higher peak concentration in the plasma and consequently a lower threshold on a mg/kg bw basis for

C_{max}-driven effects). However, it is not known whether the animals in the acute toxicity study were fasted. Another factor contributing to the difference in general toxicity may be precipitation of part of the barium cations by sulphate anions present in the diet to form insoluble barium sulphate, for which oral absorption is negligible (ATSDR, 2007). Nevertheless, dietary levels of sulphate are typically low.

Nine out of 10 top dose males showed severe aspermatogenesis (8 males complete absence of spermatogonial-type cells in tubules, 1 male less than 5% of tubules containing spermatogonia). Epididymal tubules of these nine animals did not contain any spermatocytes. The remaining top dose male showed mild aspermatogenesis and small testes. No effect on reproductive organs was observed in females (parameters examined: histopathology in the control and high dose group, ovary weight).

***Table**: 90-day study with barium metaborate (Study report, 1993a): testicular findings*

Dose (ppm)	**0**	**1000**	**5000**	**10000**
Dose (mg/kg bw/d)	0	70	349	707
No. of animals examined	10	8	10	10
Terminal body weight (g)	480	491	477	433*
Testes weight, absolute (g)	3.54	3.48	3.58	1.39**
Testes weight, relative to bw (%)	0.743	0.712	0.754	0.317**
Testes: aspermatogenesis	0	0	0	10 (1 mild, 9 severe)
Epididymides: no spermatocytes in tubules	0	0	0	9

* Statistically significant difference from control: *, $p<0.05$; **, $p<0.01$

Studies with boric acid and borax

Key animal studies investigating the effects of boric acid and borax on fertility and/or reproductive organs are summarised in the table below (further details can be found in the CLH report and its annex). The dose levels in boron equivalents are converted to equivalent doses of barium metaborate and barium chloride in order to facilitate estimation of general toxicity.

***Table**: Overview of key animal studies investigating the effects of boric acid and borax on fertility and/or reproductive organs*

Study type, species; substance; reference	**Dose boron (mg/kg bw/d)**	**Equiv. dose $Ba(BO_2)_2$ / $BaCl_2$ (mg/kg bw/d)**	**Effect(s) related to fertility**	**General toxicity (boric acid or borax)**
90-day, rat, dietary Boric acid, borax Weir and Fisher, 1972	158	1600 / 1500	Males: complete testes atrophy (all animals), reduced testes weight (absolute by 76%/77%, relative by 56%/53% b.a./borax) Females: reduced ovary	Clinical signs, reduced bw (males by 44%/55%, females by 12/10% b.a./borax)

			weight (absolute by 27%/42%, relative by 16%/32% b.a./borax)	
	47	490 / 450	Males: partial testes atrophy (5 animals out of 20?)	None
90-day, dog, dietary Boric acid, borax Weir and Fisher, 1972	44	450 / 420	Males: severe testicular atrophy and complete degeneration of the spermatogenic epithelium (all animals), reduced testes weight (absolute by 39%/44% b.a./borax)	No effect on bw, no clinical signs
3-generation, rat, dietary Boric acid, borax Weir and Fisher, 1972	59	600 / 560	All parent groups were found to be sterile. Only 1 out of 16 females produced a litter when mated with control males. Males: testes atrophy and lack of viable sperm (all animals) Females: decreased ovulation in approx. half of the examined ovaries	Reduced bw (data not shown)
9-week, investigation of testicular toxicity, rat, dietary Boric acid Ku *et al.*, 1993	68	700 / 650	From week 2 severely inhibited spermiation, from week 6 complete atrophy (> 95% atrophic tubules); from week 3 to 9 reduced testis weight (by up to 68%), testicular sperm count (by up to 99%), epididymis weight (by up to 57%) and epididymal sperm count (by up to 97%); increased FSH and LH	Reduced bw by 16%
	38	390 / 370	From week 2 severe and widespread inhibition of spermiation; from week 4 to 9 reduced epididymal sperm count (by up to 97%) and epididymis weight (by up to 29%); increased FSH	No effect on bw
4-week, investigation of testicular toxicity, rat, dietary Boric acid Treinen and	189	1900 / 1800	Inhibited spermiation, peripheral spermatid nuclei, epithelial disorganisation, cell exfoliation, luminal occlusion, cell death, significant loss of	Reduced bw by 8%

Chapin, 1991			spermatocytes and spermatids from all stage tubules (all animals); decreased basal testosterone level (by 69%)	
Continuous breeding, mouse, dietary Boric acid Fail *et al.*, 1991	221	2300 / 2100	None of the F0 pairs was fertile Males: marked tubular atrophy (many tubules Sertoli cell-only), reduced testis weight (by 86%), reduced no. of spermatids/testis (by 65%), 12/15 males had no sperm in the epididymides	Reduced bw (by ca. 16%/10% males/females)
	111	1100 / 1100	Fertility index for the 1st mating unaffected but then progressively decreased down to 5% for the 4th and 5th mating; crossover mating showed that males were the affected sex Males: tubular degeneration, reduced testis weight (by 51%), reduced epididymis and prostate weight, reduced epididymal sperm count (by 72%), decreased percentage of motile sperm, increased percentage of abnormal sperm (ca. 5-fold)	Reduced bw (females by 7%)
60-day, investigation of male fertility, rat, dietary Borax Lee *et al.*, 1978	100	1000 / 960	Reduced testis weight (by 62%) and epididymis weight (by 37%); most germinal elements absent, decreased seminiferous tubular diameter; increased FSH (2.8-fold) Serial mating with untreated females: reduced pregnancy rate till week 4 post-exposure	None

The table provides information on general toxicity in the studies themselves. However, should similar studies be conducted with barium metaborate, some of the higher doses would probably lead to marked general toxicity including mortality due to the toxicity of the barium cation. Excessive mortality makes concurrent reproductive effects less relevant for classification (cf. CLP, Annex I, 3.7.2.4.4).

General toxicity due to barium in rat dietary studies can be estimated from the 90-day study with barium metaborate (Study report, 1993a) where a dose of ca. 700 mg/kg bw/d caused a 10% body weight reduction but no clinical signs or mortality in males. Thus, the reproductive effects in the 3-generation study by Weir and Fisher (1972) at 59 mg B/kg bw/d and the testicular findings in the 9-week study by Ku *et al.* (1993) at 38 mg B/kg bw/d provide additional support regarding an adverse impact of barium metaborate on male fertility.

General toxicity due to barium in the mouse generational study by Fail *et al.* (1991) is difficult to estimate. No mouse dietary studies with barium metaborate or barium chloride are available. Drinking water studies with barium chloride (NTP, 1994) suggest that the threshold for mortality in mice is approx. 2-fold higher than in rats, so at least the dose of 1100 mg/kg bw/d could be tolerated; however, this estimate is associated with substantial uncertainty.

No data are available to inform about general toxicity of barium to dogs, so the testicular findings in the dog study by Weir and Fisher (1972) are of unclear relevance for classification of barium metaborate.

Interestingly, the 3-generation study in rats by Weir and Fisher (1972) reported also an effect on female fertility (only one out of 16 females at 59 mg B/kg bw/d produced a litter after mating with control males).

Human data

A cross-sectional study by Duydu *et al.* (2018a) found no association between blood boron levels and semen parameters or hormone levels (FSH, LH, total testosterone) in a group of subjects occupationally exposed to borates in Turkey. Mean blood boron level in the extreme exposure group was 0.57 µg/g. An earlier study by the same research group was also negative at a lower maximum exposure level (Duydu *et al.*, 2011). For comparison, Ku *et al.* (1993) reported mildly inhibited spermiation in a group of rats administered boric acid with mean serum boron level of 6.7 µg/g.

The remaining epidemiology studies on fertility presented in the CLH report were negative as well. The exposure levels, where they could be estimated, appear to have been below ca. 1-2 mg B/kg bw/d while the NOAEL for fertility in the rat is ca. 18 mg B/kg bw/d (Weir and Fisher, 1972). Most of the presented studies had limitations affecting their reliability and sensitivity (e.g. self-reporting, small sample size, lack of exposure measurements).

Overall, the negative human data do not contradict the positive animal data since the highest exposure levels in epidemiology studies were still well below the animal LOAELs and some of the epidemiology studies had significant limitations. This conclusion is in line with the RAC opinion on boric acid (2014).

Conclusion on classification

Classification in Category 1B is justified based on severe aspermatogenesis in the absence of marked general toxicity in a 90-day rat study with barium metaborate (Study report, 1993a). Rat studies with boric acid or borax reporting adverse effects on male reproductive organs and/or fertility provide additional support for classification of barium metaborate (Weir and Fisher, 1972; Ku *et al.*, 1993). There is also some evidence of adverse effect on female fertility in rats (Weir and Fisher, 1972). The negative epidemiology studies in males exposed to boric acid and borates do not contradict the animal data due to exposure levels well below the animal LOAELs and due to methodological limitations.

Specific concentration limit

SCLs are derived according to the procedure described in the CLP guidance. Classification of barium metaborate in Category 1B for fertility is based mainly on aspermatogenesis in the 90-day study with barium metaborate (Study report, 1993a). All (10 out of 10) animals were affected at 707 mg/kg bw/d while no effect was observed at the next lower dose of 349 mg/kg bw/d. The ED_{10} obtained by linear interpolation is 385 mg/kg bw/d, corresponding to the medium potency group (4 mg/kg bw/d < ED_{10} < 400 mg/kg bw/d). The value is close to the border with low potency group but no modifying factor reducing the concern has been identified. Thus, the final potency group is 'medium' and the generic concentration limit (GCL) of 0.3% applies.

Adverse effects on development

PNDT study in rabbits with barium metaborate (Study report, 1993b)

The study was carried out according to an US EPA guideline and under GLP. Pregnant New Zealand rabbits were administered barium metaborate (Busan 11-M1) in aqueous methyl cellulose via gavage from GD 7 to 19. The top dose of 20 mg/kg bw/d was chosen based on a preliminary experiment where doses from 20 to 160 mg/kg bw/d caused mortality (100% mortality from 125 mg/kg bw/d) and clinical signs of toxicity. One animal died at 20 mg/kg bw/d also in the main study (on GD 16) and another top dose dam aborted on GD 22 (preceded by hypoactivity on GD 20-21). No developmental toxicity was observed in the main study. No developmental toxicity was reported in the range-finding study (7 dams per group) at the doses available for evaluation (up to 90 mg/kg bw/d; no visceral or skeletal examination).

Studies with boric acid

Key animal studies investigating developmental toxicity of boric acid are summarised in the table below (further details can be found in the CLH report and its annex).

***Table**: Overview of developmental effects in studies with boric acid*

Study type, species; reference; dosing period	**Dose boron (mg/kg bw/d)**	**Equiv. dose $Ba(BO_2)_2$ / $BaCl_2$ (mg/kg bw/d)**	**Developmental effects**	**Maternal toxicity (boric acid)**
PNDT, rat, dietary Heindel *et al.*, 1992 Dosing GD 0-20 except for 94 mg B/kg bw/d, which was dosed GD 6-15	94	970 / 910	Increased resorptions (36% vs. 4% in controls), reduced litter size (9.7 vs. 15.4 in controls), reduced foetal weight (by 52%); increased incidence of curly/short tail, anophthalmia, microphthalmia, displaced eye, convoluted retina, enlarged lateral ventricles, cardiovascular malformations, agenesis of rib XIII, short rib XIII, fused ribs, cleft sternum; reduced ossification	None
	58	600 / 560	Reduced foetal weight (by 37%); increased incidence of enlarged lateral ventricles, agenesis of rib XIII, short rib XIII, cleft sternum, clubbed	None

			limb; wavy rib, reduced ossification	
	29	300 / 280	Reduced foetal weight (by 13%); increased incidence of short rib XIII; wavy rib	None
PNDT, rat, dietary Price *et al.*, 1996a (follow-up study to Heindel *et al.*, 1992) Dosing GD 0-20; termination: Phase I GD 20, Phase II PND 21	25	260 / 240	Phase I: reduced foetal weight (by 13%); short rib XIII; wavy rib Phase II: short rib XIII	None
PNDT, mouse, dietary Heindel *et al.*, 1992 Dosing GD 0-17	175	1800 / 1700	Increased resorptions (19% vs 6%), reduced foetal weight (by 33%); increased incidence of short rib XIII	Renal tubular dilation; corrected bw not affected
PNDT, rabbit, gavage Price *et al.*, 1996b Dosing GD 6-19	44	450 / 420	Increased resorptions (90% vs 6%); 11 out of 14 surviving foetuses were malformed (mainly cardiovascular malformations)	Reduced food consumption (by 31% GD 6-19)
	22	230 / 210	None	None
Continuous breeding, mouse, dietary Fail *et al.*, 1991	111	1100 / 1100	Reduced litter size (may be partly due to a severe effect on spermatogenesis), decreased percentage of pups born alive (88% vs 99%), reduced pup weight (by 14%)	Reduced bw (by 7%)

As stated previously, should similar studies be conducted with barium metaborate, some of the higher doses would probably lead to marked maternal toxicity including mortality due to the toxicity of the barium cation.

The rat dietary study by Heindel *et al.* (1992) reported malformations at doses equivalent to 970 and 600 mg/kg bw/d barium metaborate. Maternal toxicity of barium metaborate can be estimated from the 90-day study (Study report, 1993a), where non-pregnant females showed a 10% body weight reduction but no clinical sings or mortality at approx. 800 mg/kg bw/d. Thus, at least the dose of 58 mg B/kg bw/d (equiv. to 600 mg/kg bw/d barium metaborate) causing agenesis of rib XIII and severe foetal weight reduction (by 37%) is considered relevant for classification. Although pregnant animals might be more sensitive, the available evidence for higher sensitivity of pregnant rats to barium toxicity compared to non-pregnant ones is not very strong (see 'RAC general comment'). Short rib XIII (less than half the length of rib XII, see Price *et al.*, 1996a) appears to be a grey-zone anomaly but the high incidence in the absence of maternal toxicity and morphological relation to rib XIII agenesis (a malformation) raise a concern.

***Table**: Developmental findings in the rat PNDT study with boric acid by Heindel et al. (1992) (reduced ossification and wavy ribs omitted)*

	Dosing GD 0-20				**Dosing GD 6-15**	
Dose (ppm)	**0**	**1000**	**2000**	**4000**	**0**	**8000**
Dose (mg B/kg bw/d)	0	14	29	58	0	94
Equivalent dose of barium metaborate (mg/kg bw/d)	0	140	300	600	0	970
No. of pregnant dams	28	28	26	26	14	14
% Resorptions/litter (±SD)	3.5 (±1.0)	5.9 (±1.2)	3.4 (±0.8)	8.6 (±3.9)	4.4 (±1.9)	36.2* (±8.7)
No. of live foetuses/litter	15.4 (±0.4)	15.4 (±0.5)	15.7 (±0.4)	15.4 (±0.5)	15.4 (±0.7)	9.7* (±1.6)
Foetal weight, males (g)	3.8	3.6*	3.3*	2.4*	3.8	1.8*
Foetal weight, females (g)	3.6	3.4*	3.1*	2.3*	3.6	1.8*
No. of foetuses examined	431	432	408	386	215	136
Curly tail and/or short tail (no. of foetuses affected)	0	0	0	0	0	15
Anophthalmia	1	0	0	0	1	6
Microphthalmia	0	0	0	1	0	7
Enlarged lateral ventricles of the brain	0	0	0	21	0	24
Displaced eye	0	0	0	0	0	7
Convoluted retina	0	0	0	0	0	9
Pulmonary artery and aorta arise from right ventricle	0	0	0	0	0	5
Transposition of aorta and pulmonary artery	0	0	0	0	0	2
Other pulmonary artery malformations	0	0	0	0	0	5
Interventricular septal defect	0	0	0	0	0	3
Agenesis of rib XIII	1	1	0	24	0	17
Short rib XIII	1	11	28	152	1	50
Fused ribs	0	0	0	0	0	6
Cleft sternum	0	0	4	8	0	13
Clubbed limb (without bone change)	0	0	0	8	0	3

* Statistically significant difference from control, $p < 0.05$; incidences of anomalies not subject to statistical analysis

High embryolethality and teratogenicity were also observed in a rabbit gavage PNDT study with boric acid at 44 mg B/kg bw/d (Price *et al.*, 1996b) while no teratogenicity was seen at 22 mg/kg bw/d. The corresponding doses of barium metaborate are 450 mg/kg bw/d and 230 mg/kg bw/d, respectively. RAC noted that 20 mg/kg bw/d was chosen as an appropriate top dose in a rabbit

PNDT study with barium metaborate in the same strain and of a comparable design (Study report, 1993b) based on maternal mortality in a preliminary study (100% mortality from 125 mg/kg bw/d, high mortality already after a single dose at 160 mg/kg bw/d). Thus, it appears that developmental toxicity in rabbits cannot be achieved with barium metaborate via gavage administration. It cannot be excluded that developmental toxicity would be observed in a dietary study with barium metaborate in rabbits, but without an actual study this remains a speculation.

Regarding mice, the dose of barium metaborate (1800 mg/kg bw/d) equivalent to the developmentally toxic dose in the mouse PNDT study by Heindel *et al.* (1992) is well above the limit dose of 1000 mg/kg bw/d and thus of limited relevance for classification. In addition, high maternal toxicity cannot be excluded.

Human data

A retrospective study by Duydu *et al.* (2018b) found no association between blood boron levels and pregnancy outcome in women exposed to boron via drinking water in Turkey. The mean blood boron concentration in the high exposure group was 0.27 µg/g. The study had several limitations (self-reporting, low sample size, boron levels measured only after birth). For comparison, the mean blood boron level at the rat developmental NOAEL (9.6 mg B/kg bw/d) was 1.3 µg/g (Price *et al.,* 1996a; 1997).

A prospective cohort study by Igra *et al.* (2016) found a statistically significant inverse association between serum boron levels during pregnancy above 0.08 µg/mL and birth length (but not birth weight) in women and their offspring exposed to boron via drinking water in Argentina. The authors indicated a possible contribution of lithium, whose blood concentration was highly correlated with serum boron concentration, to the observed effect.

The remaining human studies presented by the DS were negative but had significant limitations. Overall, the epidemiology data are not considered to contradict the positive animal data.

Conclusion on classification

Classification in Category 1B for development is considered justified mainly based on malformations in the rat PNDT study with boric acid, by Heindel *et al.* (1992). Markedly increased incidence of agenesis of rib XIII was observed from 58 mg B/kg bw/d. The equivalent dose of barium metaborate (600 mg/kg bw/d) would probably not cause excessive maternal toxicity. Other findings at this dose, such as short rib XIII, clubbed limb, cleft sternum, enlarged lateral ventricles and markedly reduced foetal weight (by 37%) provide additional support for classification. The available epidemiology data for borates, indicating no or minimal developmental effects, are not considered to contradict the animal data due to low exposure levels and due to methodological limitations in some of the studies.

Specific concentration limit

Classification of barium metaborate in Category 1B for development is based mainly on malformations and grey-zone anomalies in the rat PNDT study with boric acid by Heindel *et al.* (1992). Quantitative read-across is associated with some uncertainty due to toxicokinetic factors (rate of conversion of barium metaborate to boric acid), but the impact is assumed not to be substantial (see the section on read-across). Immediate conversion of barium metaborate to boric acid in the stomach will be assumed for the purpose of SCL derivation as worst case. ED_{10} values and LOAELs (converted to equivalent doses of barium metaborate) for the relevant effects are shown in the table below. Only foetus-based incidences of the individual malformations and anomalies are available in the publication.

Effect	ED_{10} (mg/kg bw/d)	LOAEL (mg/kg bw/d)
Agenesis of rib XIII	n.a.[a]	600
Short rib XIII	330	140[b]
Cleft sternum	n.a.[a]	600[c]
Clubbed limb (without bone change)	n.a.[a]	600
Enlarged lateral ventricles of the brain	n.a.[a]	600

[a] rate of 10% not reached

[b] a similar LOAEL was identified also in the follow-up study Price *et al.* (1996a)

[c] the increase at 2000 ppm boric acid (equivalent to 300 mg/kg bw/d barium metaborate) observed in study Heindel *et al.* (1992) was not confirmed in the follow-up study Price *et al.* (1996a)

The only structural effect in the medium potency range (4 mg/kg bw/d < ED_{10} < 400 mg/kg bw/d) is short rib XIII. There seems to be a low level of agreement as to whether this finding should be considered a malformation (cf. RAC opinion on boric acid and borates, 2019) and consequently it is not clear whether short rib XIII would lead to classification in Category 1B should this be the only developmental effect observed. However, short rib XIII is structurally related to agenesis of rib XIII, clearly a malformation, the incidence of which was increased from 600 mg/kg bw/d (as barium metaborate equivalent) in this study. Therefore, increased incidence of short rib XIII to more than 10% (in the medium potency range) is considered a finding of relatively high concern in this case. Consequently, the final potency group is 'medium' and the GCL of 0.3% applies.

Adverse effects on or via lactation

No information related to effects on or via lactation is available for barium metaborate or other barium salts. A 3-generation rat study with borax (Weir and Fisher, 1972) reported reduced survival of F3A pups (1st mating) by 14% at weaning; no effect was observed in F3B pups (2nd mating) or in the groups administered boric acid. Therefore, the finding in F3A pups administered borax is not considered treatment-related. No significant reduction in pup weight or viability attributable to an effect on or via lactation was observed in the mouse multigeneration study by Fail *et al.* (1991).

Boron levels of 10-285 mg/kg were found in breast milk of mothers administered 1-13 g of boric acid (publication from 1972, cited by Moseman, 1994). The available information does not allow to conclude whether these boron levels in breast milk are potentially toxic.

Conclusion on classification

The available information does not meet the criteria for classification for adverse effects on or via lactation.

Overall conclusion on reproductive toxicity

RAC agrees with the DS's proposal of **Repr. 1B; H360FD without a specific concentration limit**. The classification for fertility is based mainly on severe effects on spermatogenesis in a study with barium metaborate. The classification for development is based on read-across from boric acid, taking into account the general toxicity of the barium cation. Skeletal malformations and anomalies in the rat have been identified as the most relevant effect with regard to developmental toxicity classification of barium metaborate. RAC also agrees with the DS's proposal of **no classification for effects on or via lactation**.

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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).