Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Trimethyl borate

EC Number: 204-468-9
CAS Number: 121-43-7

CLH-O-0000007155-76-01/F

Adopted

15 September 2022

15 September 2022

CLH-O-0000007155-76-01/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: **Trimethyl borate**

EC Number: **204-468-9**

CAS Number: **121-43-7**

The proposal was submitted by **The Netherlands** and received by RAC on **5 August 2021.**

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

PROCESS FOR ADOPTION OF THE OPINION

Netherlands 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 **20 September 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **19 November 2021**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ifthekhar Ali Mohammed**

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 **15 September 2022** by **consensus**.

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

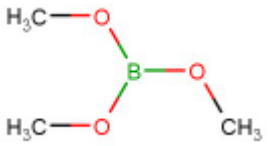
	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	005-005-00-1	Trimethyl borate	204-468-9	121-43-7	Flam. Liq. 3 Acute Tox. 4*	H226 H312	GHS02 GHS07 Wng	H226 H312			
Dossier submitters proposal	005-005-00-1	Trimethyl borate	204-468-9	121-43-7	**Add** Repr. 1B	**Add** H360FD	**Add** GHS08 **Modify** Dgr	**Add** H360FD			
RAC opinion	005-005-00-1	Trimethyl borate	204-468-9	121-43-7	**Add** Repr. 1B	**Add** H360FD	**Add** GHS08 **Modify** Dgr	**Add** H360FD			#
Resulting Annex VI entry if agreed by COM	005-005-00-1	Trimethyl borate	204-468-9	121-43-7	Flam. Liq. 3 Acute Tox. 4* Repr. 1B	H226 H312 H360FD	GHS02 GHS07 GHS08 Dgr	H226 H312 H360FD			#

#The inclusion of a specific note to apply additivity for boron compounds that exert their reproductive toxicity through the same toxic entity (boric acid/borate ion) is supported by RAC.

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Trimethyl borate (synonym: boric acid trimethyl ester) is a colourless liquid at room temperature with a boiling point of ca. 68 °C and a vapour pressure of 1.48 x 10^4 Pa (at 20 °C).



Structural formula of trimethyl borate

Uses at industrial sites and by professional workers are identified in the REACH full registration dossier(s). It is used in welding and soldering products, biocides and plant protection products, and as a laboratory chemical. It is also registered as an intermediate under REACH.

One mole of trimethyl borate hydrolyses rapidly in water to 1 mole of boric acid and 3 moles of methanol (Steinberg et al., 1957). Although there are no studies available under physiological conditions, it is plausible that trimethyl borate hydrolyses to boric acid and methanol also under such conditions. The registrants of the REACH registration dossier(s) and the dossier submitter (DS) of the CLH report used the data on boric acid and methanol to read-across to trimethyl borate for reproductive toxicity hazard (the only hazard class addressed in the current CLH dossier). The WHO-IPCS report (1998) on boron states that the chemical and toxicological properties of boric acid and other borates are expected to be similar on a mol boron/litre equivalent basis when dissolved in water or biological fluids at the same pH and low concentration. RAC has previously accepted read-across between boric acid and other borates in its CLH opinions on:

- Boric acid (RAC, 2014b), CLH-O-0000003738-64-03/F; classified as Repr. 1B; H360FD.
- Disodium octaborate anhydrate (RAC, 2014c), CLH-O-0000003654-72-03/F; classified as Repr. 1B; H360FD.
- Disodium octaborate tetrahydrate (RAC, 2014d), CLH-O-0000003655-70-03/F; classified as Repr. 1B; H360FD.
- Boric acid, diboron trioxide, tetraboron disodium heptaoxide hydrate, disodium tetraborate anhydrous, orthoboric acid sodium salt, disodium tetraborate decahydrate and disodium tetraborate pentahydrate (RAC, 2019), CLH-O-0000001412-86-300/F; removed existing SCLs for reproductive toxicity hazard class resulting in GCL of 0.3% to apply.

Therefore, RAC considers the read-across of data on boric acid and methanol to trimethyl borate as valid.

There is also a RAC opinion on methanol (RAC, 2014a) (CLH-O-0000004421-84-03/F) assessing developmental toxicity with the conclusion that there is not sufficient evidence for classifying methanol for this hazard.

Toxicokinetics

Trimethyl borate undergoes rapid and complete hydrolysis in water (Steinberg et al., 1957). This is supported by the OECD TG 111 (hydrolysis as a function of pH) data available on other alkoxy

borates (tris(2-ethylhexyl) orthoborate; tris[2-(2-hydroxyethoxy)ethyl]borate; triboron trimethyl hexaoxide) that showed complete and rapid (<10 or 5 min) hydrolysis in water.

Boric acid is completely absorbed (close to 100%) via oral and inhalation routes and the absorption via dermal route is negligible. Boric acid is distributed rapidly, not further metabolised due to the strong B-O bond and is excreted via urine (REACH registration dossier(s)).

Methanol is readily absorbed through oral, inhalation and dermal routes and distributed uniformly to all organs and tissues according to the water content (REACH registration dossier(s)). There are differences in metabolism of methanol between rodents and humans. The first step in the metabolism of methanol is mediated by catalase and alcohol dehydrogenase in rodents and by alcohol dehydrogenase in primates. The rate-limiting step in rodents is the formation of formic acid, whereas in primates it is the further degradation of formic acid. This results in methanol accumulating in the blood of rodents, while formic acid and methanol accumulate in human blood. In the human population, it is known that polymorphism in alcohol dehydrogenase exist, leading to differences in sensitivity to methanol both at the individual level and also at the population level. It can be speculated that sensitivity to methanol toxicity is also affected by such polymorphisms (RAC, 2014a).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

There are no reproductive toxicity studies with trimethyl borate and it does not have harmonised classification for reproduction hazard class. The DS proposed Cat. 1B for both fertility and developmental toxicity for trimethyl borate based on data on its hydrolytic products, boric acid and methanol. The DS considered the information on boric acid and other borates from previous CLH-dossiers on these substances and also included new studies on effects of boric acid published in 2019 and 2020. For methanol, the DS considered the information reviewed by the Health Council of the Netherlands (2006) and the US EPA (2013), the information available in the REACH registration dossier(s) and the CLH-dossier on methanol.

In line with the RAC opinion on boric acid and borates (RAC, 2019), the DS considered that no SCL is justified for reproductive toxicity of trimethyl borate and thus, the GCL of 0.3% should apply.

The DS proposed no classification for trimethyl borate for adverse effects on or via lactation due to lack of data.

Comments received during consultation

Only one MSCA commented during the consultation and supported the proposal by the DS but considered that the read-across could be further elaborated. They also pointed out that in the Steinberg et al. (1957) study the hydrolysis of trimethyl borate was not under "physiological conditions" and was not tested in 'pure water' but rather under 'conditions of possible applications' (a solution of 50 mL water, 5 gms mannitol, 4 drops of phenolphthalein and one half the amount of 0.247 or 0.1130 N sodium hydroxide).

The DS responded that the conditions in the Steinberg et al. (1957) study were indeed not physiological, but the study did demonstrate that trimethyl borate is rapidly hydrolysed when

dissolved in water. The DS also cited the Merck Index monograph on trimethyl borate which states that it hydrolyses in the presence of water to methanol and boric acid. In further support of hydrolysis of trimethyl borate, the DS presented the OECD TG 111 (hydrolysis as a function of pH) data available on other alkoxy borates that were completely and rapidly hydrolysed in water.

Assessment and comparison with the classification criteria

There are no human data or animal studies relevant to reproductive toxicity on trimethyl borate. The assessment is therefore based on data on its hydrolysis products, boric acid and methanol.

Adverse effects on sexual function and fertility

Information on boric acid and other borates

In the RAC opinions on boric acid and other borates that are all classified in Cat. 1B for fertility effects (and developmental toxicity), RAC concluded that the reproductive toxicity and repeated dose toxicity studies in mice, rats and dogs clearly indicate that borates impair fertility through atrophy and seminiferous tubule degeneration in the testes. The effects observed in the different species are similar in nature. Based on the data from the 2-year feeding study on boric acid in rats (Weir, 1996a – as cited in RAC, 2019), the overall NOAEL for fertility is 100 mg/kg bw/d, equivalent to 17.5 mg/kg bw/d of boron. This conclusion on the testicular effects and the overall NOAEL is also supported by the study conducted with disodium tetraborate decahydrate (Weir, 1996b – as cited in RAC, 2019). As the incidence of animals with testis atrophy was increased by 10% at the NOAEL, RAC concluded that this value is also the ED_{10} (100 mg boric acid/kg bw/d, equivalent to 17.5 mg boron/kg bw/d) for this effect (see Table 1 below).

***Table 1**: Incidence of testis atrophy after chronic exposure to boric acid in the Weir (1996a) study*

Dose mg boric acid (boron)/kg bw/d	0 (0)	33 (5.9)	100 (17.5)	334 (58.5)
No. of animals	3/10	1/10	4/10	10/10

In a new sub-acute study (Aktas et al., 2020), boric acid was administered for 4-6 weeks by gavage to 10 male mice per dose group at boron equivalent doses of 0, 20.1, 43.8 and 78.8 mg/kg bw/d. There were no effects on testicular weights but statistically significant increase in DNA damage in sperm cells, reduced sperm cell viability and motility were observed (see Table 2 below). Oxidative stress in testicular tissue was evident from decreased sperm cell membrane integrity, decreased glutathione and increased malondialdehyde levels. This study supports the male fertility effects of boric acid and other borates observed in earlier studies.

***Table 2**: Effects observed after 6-week exposure to boric acid in the Aktas et al. (2020) study*

Dose mg boric acid (boron equivalent)/kg bw/d	**DNA damaged sperm cell (% of total)**	**Live cells in sperm (% of total)**	**Sperm motility (% of total)**
0	0.00	74.0	78
115 (20.1)	3.30*	68.0*	72.5
250 (43.8)	6.20*	68.2*	68.5*
450 (78.8)	14.4*	57.0*	54.0*

*$p<0.05$, pair-wise comparison to control group

Two new studies are available where workers were exposed to boron through environmental and occupational exposure.

In a study by Duydu et al. (2019), 304 male workers were divided into different exposure groups: control (<50 ng B/g blood), low (50-100 ng B/g blood), medium (100-150 ng B/g blood), high (150-400 ng B/g blood) and extreme exposure group (>400 ng B/g blood). Significantly ($p<0.05$) increased boron levels were found in semen and urine of the medium, high and extreme exposure groups. No association was found between blood or semen boron levels and Y:X ratio in sperm. There was also no significant effect on sex ratio at birth in any of the exposure groups.

In a study by Basaran et al. (2019), 212 male workers were divided into different groups starting from very low exposure group (<100 ng B/g blood) to extreme exposure group (≥651 ng B/g blood). There was no correlation between blood boron levels and DNA damage in sperm and lymphocytes in any exposure groups. A significantly ($p=0.042$) lower micronucleus frequency was observed in buccal cells in only very low exposure group, but the sample size was small in this group (n=12).

The blood boron levels even in the highest exposure groups in the human studies (including the two above) correspond to < 1 mg B/kg bw/d based on an average weight of 70 kg. In contrast, the LOAELs in the animal studies for adverse effects on fertility are 26 – 58.5 mg B/kg bw/d. Therefore, as also previously concluded by RAC in the boric acid/borates opinion, the lack of fertility effects in human studies does not contradict the animal data.

Information on methanol

No human studies showing adverse effects on fertility upon exposure to methanol are available.

In a two-generation reproduction toxicity study (similar to OECD TG 416), 30 SD rats/sex/group were exposed via inhalation (whole-body) for 20 h/d to 0, 10, 100 or 1000 ppm methanol (= 0, 13, 131, 1310 mg/m^3) (Takeda, 1988). There were no effects on fertility indices in the F0 and F1 generations. Testis descent was earlier as compared to controls in F1 and F2 pups (see Table 17 on page 24 of the CLH-report).

In a one-generation reproduction toxicity study, 9 – 12 female monkeys (*Macaca fasicularis)* per group were exposed via inhalation (whole-body) for 2.5 hr/d, 7 d/week during premating (ca. 120 d), mating (ca. 65 d) and gestation (ca. 163 d) to 0, 200, 600 or 1800 ppm methanol (= 0, 262, 786, 2358 mg/m^3) (Burbacher et al., 1999). Males were not exposed in the study. There were no effects on menstrual cycles, conception rate, live-birth index. The duration of pregnancy (see Table 3 below) was significantly shorter ($p=0.04$) in all groups but was stated as still within normal range for this strain of animals (no further information is available on this effect in the CLH-report). Complications observed at delivery (vaginal bleeding without labour and long-term non-productive labour) were reported as not treatment related.

***Table 3**: Maternal weight gain during pregnancy and duration of pregnancy in Macaca fascicularis upon inhalation exposure to methanol in the Burbacher et al. (1999) study*

Exposure Group	Weight Gain[b] (kg)	Duration of Pregnancy[c] (days)
Control $n = 9$	1.67 ± 0.07 (1.33–2.05)	168 ± 2[d] (162–178)
200 ppm $n = 9$	1.27 ± 0.14 (0.51–1.76)	160 ± 2 (153–172)
600 ppm $n = 9$	1.78 ± 0.25 (1.09–3.45)	162 ± 2[d] (153–166)
1,800 ppm $n = 9$	1.54 ± 0.20 (0.52–2.31)	162 ± 2 (150–169)

[a] Values are presented as means ± SE with range in parentheses on line below.

[b] No statistically significant differences were found in maternal weight gain during pregnancy across the four methanol-exposure groups (ANOVA; $p < 0.12$, all tests).

[c] Pregnancy durations for the methanol-exposure groups were significantly shorter than that for the control group (ANOVA post hoc tests; $p = 0.04$, all tests).

[d] Live-born offspring only, $n = 8$.

In a combined chronic toxicity/carcinogenicity study (similar to OECD TG 453) (New Energy Developmental Organisation, 1987) in F344 rats and B6C3F1 mice, ca. 50 animals/sex/group were exposed to methanol via inhalation (whole body) for 19.5 hr/d, 7 d/week to 10, 100 or 1000 ppm (= 0, 13.1, 131, 1310 mg/m^3). Testicular atrophy observed in rats was stated as not treatment related and related to aging (no further information is available on this effect in the CLH-report). In mice, high dose group showed significantly ($p<0.05$) decreased testis weight (data not specified in the original report) and one animal had severe testicular atrophy.

In an oral carcinogenicity study (similar to OECD TG 451) in SD rats (Soffritti et al., 2002), 100 animals/sex/group were given methanol via drinking water at 0, 50, 500 or 2000 mg/kg bw/d. In the high dose group, a significant ($p<0.05$) increase in testicular interstitial hyperplasia (data not available), testicular adenomas and uterine sarcomas (see Table 12 on page 21 of the CLH-report) were observed.

In an experimental study by Ward et al. (1984), B6C3F1 mice exposed via gavage to methanol for 5 d at 0 (n=5) or 1000 (n=10) mg/kg bw/d caused non-significant increase in percentage of abnormal sperm morphologies (1.86 ± 0.91 vs. 1.12 ± 0.39% in control).

In a sub-acute study by Andrews et al. (1987), 3 cynomolgus monkeys (*Macaca fascicularis*)/sex/group and 5 CD rats/sex/group were exposed to methanol via inhalation (whole body) for 4 weeks (6 hr/d, 5 d/week) to 0, 500, 2000 or 5000 ppm (= 0, 655, 2620, 6550 mg/m^3). Macroscopic observations and reproductive organ weights (testes, epididymis and ovaries) showed no effects in monkeys or rats.

In two sub-acute studies by Poon et al. (1994; 1995), 15 SD rats/sex/group were exposed to methanol via inhalation (whole body) for 4 weeks (6 hr/d, 5 d/week) to 0, 393 or 3930 mg/m^3 (1994 study) and to 0 or 3275 mg/m^3 (1995 study). Histopathological examination of reproductive organs showed no effects in either study.

In two acute studies by Cooper et al. (1992), 10 male Long-Evans rats/group were exposed to methanol via inhalation (whole body) for 6 hr to 0, 262, 6550 or 13100 mg/m^3 (study 1) and for 1, 2 and 6 hr to 0 or 6550 mg/m^3 (study 2). Half of the animals were acclimated (2 weeks prior to handling) to the experimental conditions and the other half was not acclimated. Testis weights and sex hormones (LH, FSH, testosterone and prolactin) were measured just after exposure (both studies) and 18 hr after the end of exposure (study 1). There were no effects on testis weights. Significant effects were observed in the serum hormone levels in study 1 (see Figure 1 on page 26 of the CLH-report) and study 2 (see Figure 2 on page 27 of the CLH-report). However, the direction and magnitude of effects were strongly dependent on the acclimatisation status of the animals.

Lee et al. (1991) studied the effects of methanol on serum hormone levels and/or reproductive organs weights and morphology in male rats. In study 1, 9-10 SD rats/group were exposed to methanol via inhalation (whole body) for 1, 2, 4 or 6 weeks (8 hr/d, 5 d/week) at 0 or 262 mg/m^3. There were no significant effects on serum testosterone levels. Macroscopic observations and weights of testis and seminal vesicles showed no effects either. In study 2, the age dependent effect of methanol on testis morphology was studied in Long-Evans rats. 8-13 rats/group were exposed via inhalation (whole body) for 13 weeks (20 hr/d): to 7 months old animals at 0, 65.5, 262 or 1048 mg/m^3 and to 15 months old animals at 0 or 1048 mg/m^3. For both ages, no effects were observed on testis weights, gross testicular abnormalities and incidence of testicular lesions.

In a study by Cameron et al. (1984), 5 male SD rats/group were exposed to methanol via inhalation (whole body) for 1, 2, 4 or 6 weeks (8 hr/d, 5 d/week) at 0, 262, 2620 or 13100 mg/m^3. There were no dose-dependent effects in the serum levels of testosterone, FSH and LH.

In another study by Cameron et al. (1985), 5 male SD rats/group were exposed to methanol via inhalation (whole body) for 1 or 7 d (6 hr/d) at 0 or 262 mg/m^3. Serum levels of testosterone, LH and corticosterone were measured. After the 1-day exposure, testosterone levels were significantly decreased (-59%, $p<0.05$) immediately after exposure and returned to control values after 18 h (see Table 20 on page 28 of the CLH- report). No effects were observed after the 7-day exposure.

Overall, the animal studies with methanol showed no significant consistent effects on fertility. No treatment related adverse effects on fertility were observed in monkeys. Early testis descent was observed in the two-generation study with rats, but this is an effect on development of pups. Testicular and uterine tumours observed in the carcinogenicity study with rats are usually of low relevance for classification for fertility. Moreover, these tumours were observed only at a very high dose of 2000 mg/kg bw/d. In the combined chronic/carcinogenicity studies, testicular atrophy observed in rats was concluded as not treatment related, and in mice, it was only observed in one high dose animal. In several sub-acute studies in rats, the macroscopic observations and reproductive organ weights showed no effects, and there were no consistent or dose-dependent changes in the serum hormone levels in males. Anyways, due to difference in metabolism of methanol between rodents and humans, the rodent data is not relevant to humans. Methanol is acutely toxic in humans with 300 mg/kg bw reported as the minimum lethal dose, while the oral LD_{50} in rats for methanol is ≥ 5000 mg/kg bw (the CLP Guidance, ver. 5, pg. 260).

<u>Comparison with the CLP criteria for adverse effects on sexual function and fertility</u>

Substances are classified in Cat. 1A largely based on evidence from humans. Environmental and occupational exposure of humans to boron (the toxic moiety of boric acid) revealed no adverse effects on fertility. No relevant human data on fertility effects is available for methanol. Therefore, Cat. 1A is not applicable for trimethyl borate.

Substances are classified in Cat. 1B when the data provide clear evidence of an adverse effect on

sexual function and fertility in the absence of other toxic effects or if occurring together with other toxic effects and the adverse effect is considered not to be a secondary non-specific consequence of the other toxic effects.

Based on RAC (2014b), boric acid is already classified on Annex VI of CLP in Cat. 1B for adverse effects on fertility. The animal studies with methanol provide no clear evidence of an adverse effect on fertility. Therefore, the classification of boric acid should also apply to trimethyl borate. However, since methanol is acutely toxic in humans, it should be first considered whether the acute toxicity of methanol could prevent the adverse effects on fertility from boric acid.

The ED_{10} for effects on fertility of boric acid is 100 mg/kg bw/d (RAC, 2019). This ED_{10} for boric acid can be converted to an ED_{10} for trimethyl borate of 168 mg/kg bw/d (100 mg/kg bw/d * 103.9 g/mol / 61.83 g/mol = 168 mg/kg bw/d). Since the rodent data on methanol is not relevant to humans, the comparison between trimethyl borate and methanol should be done for effects in humans. Therefore, the ED_{10} for trimethyl borate of 168 mg/kg bw/d is extrapolated to humans considering the correction factor for allometric scaling (rat = 4; ECHA, 2012), resulting in a human ED_{10} of 168 / 4 = 42 mg/kg bw/d. The simultaneous exposure to methanol at the human ED_{10} would be (42 /103.9) * (3 * 32) = 39 mg/kg bw/d, as 3 moles of methanol are formed from 1 mole of trimethyl borate. This results in a clear margin between the ED_{10} for effects on sexual function and fertility in humans and the minimum lethal dose of methanol in humans (300 mg/kg bw)[1]. Therefore, the toxicity of methanol would not mask the reprotoxic effects of boric acid after exposure to trimethyl borate.

Thus, RAC agrees with the DS and concludes that **trimethyl borate warrants classification in Cat. 1B for adverse effects on sexual function and fertility** based on its hydrolytic product, boric acid.

Concentration limits

Since the ED_{10} of 168 mg/kg bw/d for trimethyl borate belongs to the medium potency group (a substance with a $4 < ED_{10} < 400$ mg/kg bw/d, according to section 3.7.2.6.3 of the CLP Guidance, ver. 5), RAC agrees with the DS and concludes that a Specific Concentration Limit is not justified for trimethyl borate for adverse effects on sexual function and fertility.

Adverse effects on development

Information on boric acid and other borates

In the RAC opinions on boric acid and other borates that are all classified in Cat. 1B for developmental toxicity (and fertility effects), RAC concluded that the developmental toxicity of boron was clearly observed in studies in rats and rabbits, the rat being the most sensitive species. Malformations consisted primarily of anomalies of the eyes, the central nervous system, the cardiovascular system, and the axial skeleton. The most common malformations were enlargement of lateral ventricles in the brain and agenesis or shortening of rib XIII. The overall NOAEL for embryotoxic/teratogenic effects was 9.6 mg boron/kg bw/d observed in a prenatal developmental toxicity with boric acid in rats. This was based on a reduction in mean foetal body

[1] The acute toxicity of methanol is not of concern even if the rat ED_{10} for effects on fertility of trimethyl borate is not corrected with allometric scaling.

weight/litter and an increased incidence in short rib XIII malformation at the LOAEL of 76 mg boric acid/kg bw/d (13.3 mg boron/kg bw/d) (Price et al., 1996 – as cited in RAC, 2019). The foetal incidence of short rib XIII malformation was 1.2% at the LOAEL and 1.5% at the highest dose (see Table 4 below). As the incidences are low, RAC has previously concluded that it is not possible to derive an ED_{10} for developmental toxicity. Therefore, in line with the CLP Guidance, the LOAEL was used to determine the concentration limits (RAC, 2019).

***Table 4**: The foetal incidence of short rib XIII malformation in the PNDT study in rats with boric acid (Price et al., 1996)*

Dose: mg boric acid (boron)/kg bw/d	Short rib XIII, GD 20 (%, fetuses/litter)
0	0.7
19 (3.3)	0.6
36 (6.3)	0.6
55 (9.6)	0.7
76 (13.3)	1.2*
143 (25)	1.5*

* $p < 0.05$, pair-wise comparison to concurrent control group.

In a new prenatal developmental toxicity study (Pleus et al., 2018), boric acid significantly decreased the mean foetal body weight in the mid- and high-dose groups. No other developmental toxicity was observed in this study in contrast to earlier studies on boric acid/borates. However, the highest dose in this study was 11 mg boric acid/kg bw/d which is well below the LOAEL of 76 mg boric acid/kg bw/d observed in the other studies.

In a new study on environmental exposure to boron in Turkey (Duydu et al., 2018b), 190 females were divided into low- (n=143), medium- (n=29), and high-exposure group (n=27). The estimated boron levels in the high-exposure group were at a mean value of 275 ng/g blood (range: 152 to 958 ng/g blood). There were no adverse pregnancy outcomes (sex ratio, preterm birth, birth weight, congenital abnormalities, abortion, miscarriage, stillbirth, early neonatal death, neonatal death and infant death).

The blood boron levels in the human studies were very low compared to the LOAEL for developmental toxicity in animal studies. Therefore, the lack of developmental toxicity in human studies do not contradict the animal data.

Information on methanol

RAC has previously concluded that there is robust evidence of developmental toxicity of methanol in rodents (such as increased resorption, skeletal and eye anomalies), but very limited indications of developmental toxicity from non-rodent species (weak effect on cognition). Overall, RAC concluded that classification based on animal studies is not warranted because methanol blood levels causing clear developmental toxicity in rodents would be acutely toxic or even lethal to humans. Therefore, methanol was not classified for developmental toxicity (RAC, 2014a).

Comparison with the CLP criteria for adverse effects on development

Substances are classified in Cat. 1A largely based on evidence from humans. Environmental and occupational exposure of humans to boron (the toxic moiety of boric acid) revealed no adverse effects on development. There is also no evidence from humans of developmental toxicity of methanol. Therefore, Cat. 1A is not applicable for trimethyl borate.

Substances are classified in Cat. 1B when the data provide clear evidence of an adverse effect on development in the absence of other toxic effects or if occurring together with other toxic effects and the adverse effect is considered not to be a secondary non-specific consequence of the other toxic effects.

Based on RAC opinion (RAC, 2014b), boric acid is already classified in Cat. 1B for adverse effects on development. In the RAC opinion on methanol (RAC, 2014a), it was concluded that classification for developmental toxicity is not warranted as methanol is acutely toxic at doses lower than that resulted in developmental toxicity in animal studies. In order to classify trimethyl borate, it should be considered whether the acute toxicity of methanol could prevent the adverse effects on development from boric acid.

The LOAEL for developmental toxicity of boric acid is 76 mg/kg bw/d. This LOAEL for boric acid can be converted to a LOAEL for trimethyl borate of 128 mg/kg bw/d (76 mg/kg bw/d * 103.9 g/mol / 61.83 g/mol = 128 mg/kg bw/d). Since the rodent data on methanol is not relevant to humans, the comparison between trimethyl borate and methanol should be done for effects in humans. Therefore, the LOAEL for trimethyl borate of 128 mg/kg bw/d is extrapolated to humans considering the correction factor for allometric scaling (rat = 4; ECHA, 2012), resulting in a human LOAEL of 128 / 4 = 32 mg/kg bw/d. The simultaneous exposure to methanol at the human LOAEL would be (32 /103.9) * (3 * 32) = 30 mg/kg bw/d, as 3 moles of methanol are formed from 1 mole of trimethyl borate. This results in a clear margin between the LOAEL for developmental toxicity in humans and the minimum lethal dose of methanol in humans (300 mg/kg bw)[2]. Therefore, the toxicity of methanol would not mask the developmental toxicity by boric acid after exposure to trimethyl borate.

Thus, RAC agrees with the DS and concludes that **trimethyl borate warrants classification in Cat. 1B for adverse effects on development** based on its hydrolytic product, boric acid.

Concentration limits

Since an ED_{10} could not be derived for developmental toxicity due to low incidence of the effects, the LOAEL for developmental toxicity is considered for determining concentration limits (RAC, 2019). The LOAEL of 128 mg/kg bw/d for trimethyl borate belongs to the medium potency group (a substance with a 4 < ED10 < 400 mg/kg bw/d, according to section 3.7.2.6.3 of the CLP Guidance, ver. 5). Therefore, RAC agrees with the DS and concludes that an SCL is not justified for trimethyl borate for adverse effects on development.

Adverse effects on or via lactation

Information on boric acid and other borates

In the CLH-report it is mentioned under the section on animal studies that "Relatively small amounts of boric acid have been detected in milk, indicating a limited risk of adverse effects on or via lactation (Beyer et al., 1983)." No further details are available in the CLH-report.

No human data is available showing boric acid/borates induced effects on or via lactation.

Information on methanol

In a study by Aziz et al. (2002), a dose of 1800 mg methanol/kg bw/d was shown to affect the brain development in pups (Wistar rats) exposed via lactation on postnatal day 1-21. However, due to differences in metabolism, the rodent data on methanol is not relevant to humans.

[2] The acute toxicity of methanol is not of concern even if the rat LOAEL for developmental toxicity of trimethyl borate is not corrected with allometric scaling.

Comparison with the CLP criteria for adverse effects on or via lactation

Classification for effects on or via lactation can be assigned based on:

a) human evidence indicating a hazard to babies during the lactation period; and/or
b) results of one- or two-generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

There are no relevant data on trimethyl borate itself, or its hydrolytic products boric acid and methanol that can be considered as fulfilling any of the three criteria above. Therefore, RAC agrees with the DS and concludes that trimethyl borate should not be classified for adverse effects on or via lactation.

Overall conclusion

RAC considers that trimethyl borate warrants **classification as Repro 1B H360FD for effects on sexual function and fertility as well as development**.

Inclusion of a Note

The European Commission is currently discussing a draft note (Note 11[3]), to be assigned to boron compounds for classification of mixtures as a reproductive toxicant based on the additivity approach which applies to substances whose hazard is due to the presence or formation of a common molecular entity (i.e., boric acid in this case). Since the reproductive toxicity of trimethyl borate is due to its hydrolytic product boric acid, RAC considers that additivity is also applicable to trimethyl borate.

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).

[3] COM draft for Note 11: The classification of mixtures as reproductive toxicant is necessary if the sum of the concentrations of individual boron compounds that are classified as reproductive toxicant in the mixture as placed on the market is ≥ 0.3 %.