# Committee for Risk Assessment

# RAC

Annex 1

**Background document**

to the 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

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

## Adopted

## 17 September 2020

# CLH report

# Proposal for Harmonised Classification and Labelling

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

# International Chemical Identification:

# Barium diboron tetraoxide

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

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

**Index Number:** Not assigned

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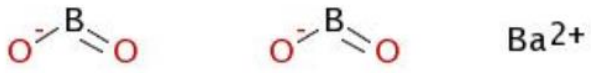
# CONTENTS

# 1 IDENTITY OF THE SUBSTANCE

## 1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

| | |
|---|---|
| **Name(s) in the IUPAC nomenclature or other international chemical name(s)** | Barium diborate |
| **Other names (usual name, trade name, abbreviation)** | Barium metaborate<br>Trade name: Busan 11-M1 |
| **ISO common name (if available and appropriate)** | *Not applicable* |
| **EC number (if available and appropriate)** | 237-222-4 |
| **EC name (if available and appropriate)** | Barium diboron tetraoxide |
| **CAS number (if available)** | 13701-59-2 |
| **Other identity code (if available)** | *Not applicable* |
| **Molecular formula** | BHO2.1/2Ba |
| **Structural formula** |  |
| **SMILES notation (if available)** | [Ba+2].[O-]B=O.[O-]B=O |
| **Molecular weight or molecular weight range** | 222.9466 |
| **Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)** | *Not applicable* |
| **Description of the manufacturing process and identity of the source (for UVCB substances only)** | *Not applicable* |
| **Degree of purity (%) (if relevant for the entry in Annex VI)** | *Not relevant* |

The current classification and labelling proposal for barium diboron tetraoxide covers all crystalline modifications of the substance.

## 1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

| **Constituent (Name and numerical identifier)** | **Concentration range (% w/w minimum and maximum in multi-constituent substances)** | **Current CLH in Annex VI Table 3.1 (CLP)** | **Current self- classification and labelling (CLP)** |
|---|---|---|---|
| barium diboron tetraoxide<br><br>EC no: 237-222-4<br>CAS no: 13701-59-2 | CONFIDENTIAL | Included in group entry, Index no. 056-002-00-7:<br><br>Acute Tox. 4*, H302<br>Acute Tox. 4*, H332 | Not Classified<br><br>Acute Tox. 4, H302<br>Acute Tox. 4, H332<br><br>Repr. 1B, H360FD<br>Skin Irrit. 2, H315<br>Eye Irrit. 2, H319<br><br>STOT SE 3, H335 Aquatic Chronic 3, H412 |

**Table 3: Impurities (non-confidential information) if relevant for the classification of the substance**

| **Impurity (Name and numerical identifier)** | **Concentration range (% w/w minimum and maximum)** | **Current CLH in Annex VI Table 3.1 (CLP)** | **Current self-classification and labelling (CLP)** | **The impurity contributes to the classification and labelling** |
|---|---|---|---|---|
| Not relevant for the classification and labelling proposal for barium diboron tetraoxide | | | | |

# 2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

## 2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 4: Proposed harmonised classification according to the CLP criteria

| | Index No | International Chemical Identification | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hazard Class and Category Code(s) | Hazard statement Code(s) | Pictogram, Signal Word Code(s) | Hazard statement Code(s) | Suppl. Hazard statement Code(s) | | |
| Current Annex VI entry | Group entry<br>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*<br>Acute Tox. 4* | H302<br>H332 | GHS07<br>Wng | H302<br>H332 | | * | A1 |
| Dossier submitters proposal | TBD | barium diboron tetraoxide | 237-222-4 | 13701-59-2 | Acute Tox. 4<br>Repr. 1B | H302<br>H360FD | GHS07<br>Dgr | H302<br>H360FD | | Oral: ATE = 530 mg/kg | |
| Resulting Annex VI entry if agreed by RAC and COM | TBD | barium diboron tetraoxide | 237-222-4 | 13701-59-2 | Acute Tox. 4<br>Repr. 1B | H302<br>H360FD | GHS07<br>Dgr | H302<br>H360FD | | | |

Table 5: Reason for not proposing harmonised classification and status under public consultation

| **Hazard class** | **Reason for no classification** | **Within the scope of public consultation** |
|---|---|---|
| **Explosives** | hazard class not assessed in this dossier | No |
| **Flammable gases (including chemically unstable gases)** | hazard class not assessed in this dossier | No |
| **Oxidising gases** | hazard class not assessed in this dossier | No |
| **Gases under pressure** | hazard class not assessed in this dossier | No |
| **Flammable liquids** | hazard class not assessed in this dossier | No |
| **Flammable solids** | hazard class not assessed in this dossier | No |
| **Self-reactive substances** | hazard class not assessed in this dossier | No |
| **Pyrophoric liquids** | hazard class not assessed in this dossier | No |
| **Pyrophoric solids** | hazard class not assessed in this dossier | No |
| **Self-heating substances** | hazard class not assessed in this dossier | No |
| **Substances which in contact with water emit flammable gases** | hazard class not assessed in this dossier | No |
| **Oxidising liquids** | hazard class not assessed in this dossier | No |
| **Oxidising solids** | hazard class not assessed in this dossier | No |
| **Organic peroxides** | hazard class not assessed in this dossier | No |
| **Corrosive to metals** | hazard class not assessed in this dossier | No |
| **Acute toxicity via oral route** | **harmonised classification proposed** | Yes |
| **Acute toxicity via dermal route** | data conclusive but not sufficient for classification | Yes |
| **Acute toxicity via inhalation route** | data conclusive but not sufficient for classification | Yes |
| **Skin corrosion/irritation** | hazard class not assessed in this dossier | No |
| **Serious eye damage/eye irritation** | hazard class not assessed in this dossier | No |
| **Respiratory sensitisation** | hazard class not assessed in this dossier | No |
| **Skin sensitisation** | hazard class not assessed in this dossier | No |
| **Germ cell mutagenicity** | hazard class not assessed in this dossier | No |
| **Carcinogenicity** | hazard class not assessed in this dossier | No |
| **Reproductive toxicity** | **harmonised classification proposed** | Yes |
| **Specific target organ toxicity-single exposure** | hazard class not assessed in this dossier | No |
| **Specific target organ toxicity-repeated exposure** | hazard class not assessed in this dossier | No |
| **Aspiration hazard** | hazard class not assessed in this dossier | No |
| **Hazardous to the aquatic environment** | hazard class not assessed in this dossier | No |
| **Hazardous to the ozone layer** | hazard class not assessed in this dossier | No |

## 3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Barium diboron tetraoxide can be found in Annex VI of the CLP Regulation under the group entry with the Index number 056-002-00-7 and following name "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 EC No 1272/2008".

### 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

Formation of a common compound

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.

## 4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

Since barium diboron tetraoxide is considered to fulfil the criteria for classification as toxic to reproduction (Repr. 1B, H360FD), a harmonised classification is thus justified according to Article 36(1)(d) of the CLP Regulation.

The proposed classification and labelling of barium diboron tetraoxide for reproductive toxicity is based on data on the substance itself, with supporting data from read-across from other tested borates (e.g. boric acid) and borate salts (borax or disodium tetraborate decahydrate). The read-across is justified because after oral exposure the substances dissociate and result in the formation of boric acid as the main species at acidic and neutral pH. The resulting classification is comparable to that of the other borates in Annex VI.

## 5 IDENTIFIED USES

Barium diboron tetraoxide is used for manufacturing of coatings and paints, thinners and paint removers in the industry, and by professional workers and consumers.

## 6 DATA SOURCES

Data from the REACH registration dossiers of barium diboron tetraoxide[1] and barium[2], from ECHA dissemination, were used. Additional information sources are represented by the CLH-report for boric acid (2013) and the respective RAC Opinion (2014).

## 7 PHYSICOCHEMICAL PROPERTIES

Table 6: Summary of physicochemical properties

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| **Physical state at 20°C and 101,3 kPa** | White crystalline solid | REACH registration (ECHA dissemination,[2018]) | |
| **Melting/freezing point** | 1367.5 °C at 101.3 kPa | REACH registration (ECHA dissemination,[2018]) | BUSAN 11-M1 fused between 1367.5 and 1482.5 °C. A weight loss of 3.6 mg (out of 31.3 mg) was observed for the sample. |

[1] Retrieved in November, 2018 at https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15812/1

[2] Retrieved in December, 2018 at https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/19625

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| **Boiling point** | No data | - | |
| **Relative density** | 0.714 ± 0.001 g/cm³ at 25 °C | REACH registration (ECHA dissemination,[2018]) | |
| **Vapour pressure** | less than 8.1 x $10^{-7}$ torr at 25 °C | REACH registration (ECHA dissemination,[2018]) | |
| **Surface tension** | No data | - | |
| **Water solubility** | 822 mg/L at 25 °C | REACH registration (ECHA dissemination,[2018]) | BUSAN 11-M1 was found to be moderately soluble in water having a solubility of 823 ppm. |
| **Partition coefficient n-octanol/water** | Pow = 0.2 at 25 °C, giving a log Pow of 0.69897 | REACH registration (ECHA dissemination,[2018]) | Based on guideline requirements the Pow was reported in the study as less than 10, giving a log Pow of less than 1. |
| **Flash point** | Not applicable | - | |
| **Flammability** | Non-flammable | REACH registration (ECHA dissemination,[2018]) | |
| **Explosive properties** | Not applicable | - | |
| **Self-ignition temperature** | Not applicable | - | |
| **Oxidising properties** | Not oxidising | REACH registration (ECHA dissemination,[2018]) | The test item/cellulose mixture did not burn when the ignition source was put in contact with the cone. The mixture carbonised and the colour changed into grey/black. Barium diboron tetraoxide is considered to be inert in terms of oxidizing properties. |
| **Granulometry** | 92.3 % of the powder has a particle size of between 0.3 and 10um | REACH registration (ECHA dissemination,[2018]) | |
| **Stability in organic solvents and identity of relevant degradation products** | No data on degradation products | - | BUSAN 11-M1 was found to be slightly soluble in octanol having a solubility of 0.38 ppm. |
| **Dissociation constant** | 8.9 at 25 °C | REACH registration (ECHA dissemination,[2018]) | |
| **Viscosity** | Not applicable | - | |

## 8 EVALUATION OF PHYSICAL HAZARDS

Physical hazards were not assessed in this dossier.

# 9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 7: Summary table of toxicokinetic studies

| Method | Results | Remarks[3] | Reference |
|---|---|---|---|
| **Human data** | | | |
| *Boric acid and borate salts* | | | |
| ***In vivo* percutaneous absorption study in humans**<br><br>Males and females aged 22 - 50 with 8 people per group were exposed to the test substance. Urine was sampled as well as T-shirts worn and skin washings sampled. | <u>*In vivo* dermal absorption</u>:<br>The absorbed dose of boric acid was 0.226 ± 0.125, with flux and permeability constants calculated at 0.0094 µg/cm$^2$/h and 1.9 x 10$^{-7}$ cm/h, respectively.<br><br>Borax (disodium tetraborate decahydrate) percent dose absorbed was 0.210 ± 0.194, with flux and permeability constants calculated at 0.00875 µg/cm$^2$/h and 1.8 x 10$^{-7}$ cm/h, respectively.<br><br>Disodium octaborate tetrahydrate absorbed dose was 0.122 ± 0.108, with flux and permeability constants calculated at 0.010 µg/cm$^2$/hr and 1.0 x 10$^{-7}$ cm/h, respectively. | Test material: boric acid, disodium tetraborate decahydrate, disodium octaborate tetrahydrate<br><br>Purity: unknown<br><br>Reliability: 1 (reliable without restriction). | Wester et al. 1998a |
| **Percutaneous absorption through human skin *in vitro***<br><br>*In vitro* diffusion from aqueous solution was determined in receptor fluid accumulation over a 24h period. Human cadaver skin (dermatomed) was clamped onto an AMIE Systems in-line cell in a flow-through apparatus, with 1 cm$^2$ surface area of skin exposed. Receptor fluid was pumped at a rate of 3 mL/hr and collected every 4 h to 24 h. After 24 h the skin surface was washed.<br><br>Boric acid (enriched) was applied at 0.05 %, 0.5 % and 5 % and either an infinite dose of 1000 mL/ cm2 or a finite dose of 2 mL/ cm2.<br><br>Changes in boron isotope ratios by IPCMS (Inductively Coupled | <u>Dermal absorption</u>:<br>The absorbed doses of boric acid were 1.2 for 0.005 % dose, 0.28 for 0.5 % dose and 0.70 % for 5 % dose. These absorption amounts translated into flux values of 0.25, 0.58 and 14.58 mg/cm$^2$/h and permeability constants (Kp) of 5.0 x 10-4, 1.2 x 10$^{-4}$ and 2.9 x 10$^{-4}$ /cm/hr. The above doses were at a standard 1000 µL/cm$^2$ dosing solutions. When the 5 % dose was applied at 2 µL/cm2 (in vivo dosing volume), flux decreased some 200-fold to 0.07 mg/cm$^2$/hr and Kp of 1.4 x 10 $^{-6}$ cm/hr.<br><br>Borax (disodium tetraborate decahydrate) dosed at 5 %/1000 µL/cm$^2$ had 0.41 % dose absorbed. Skin surface wash recovery was 87.7 ± 5.9 % dose. Flux was 8.5 µg/cm$^2$/h, and Kp was 1.7 x 10$^{-4}$ cm/h.<br><br>Disodium octaborate tetrahydrate dosed at 10 % /1000 µL/cm$^2$ was 0.19 % dose absorbed. Skin surface wash recovery was 91.3 ± 25.2 % dose. Flux was 0.8 x 10$^{-4}$ cm/h.<br><br>These *in vitro* results from infinite dose (1000 µL) were several magnitudes higher than those obtained *in vivo*. The results from the finite dose (2 µL) were closer to the *in vivo* results (also 2 µL). | Test material: boric acid, disodium tetraborate decahydrate, disodium octaborate tetrahydrate<br><br>Purity: unknown<br><br>Reliability: 1 (reliable without restriction). | Wester et al. 1998b |

[3] Where applicable and unless stated otherwise, the reliability scores of the studies presented in Table 7 are according to the CLH dossier of boric acid, assessed by RAC in 2013.

| Method | Results | Remarks[3] | Reference |
|---|---|---|---|
| Plasma-Mass Spectrometry) were used to measure absorption. | | | |
| **Dermal absorption in infants**<br><br>The plasma boron content in 22 newborn infants was assessed, following repeated daily applications of a water-emulsifying ointment containing the equivalent of 3 % boric acid to the napkin region; 3 g ointment administered in total to each infant, corresponding to 90 mg boric acid (equivalent to 15.7 mg boron). | The mean plasma-boron concentration decreased over a 5 days period, from a pre-treatment value of 0.49 to 0.29 mg/L, the corresponding values in ten untreated neonates being 0.62 and 0.21 mg/L, respectively. | Test material: boric acid<br><br>Purity: unknown<br><br>Reliability: 2 (reliable with restrictions). | Friis-Hansen et al. 1982 |
| *Boron* | | | |
| **Literature review of published and proprietary data** | Absorption: inhaled boron is absorbed and systemically distributed, almost complete gastrointestinal absorption following oral exposure.<br><br>Distribution: widely distributed throughout the body including reproductive tissues, but has a low affinity for fat. At high doses, boron accumulates in the bone.<br><br>Metabolism: being an inorganic element, boron is not metabolised by humans, but the parent borate is recovered in the blood, tissues and urine.<br><br>Elimination and excretion: excretion primarily through renal elimination; over 93% of the inhaled and ingested dose is excreted in the urine; a calculated mean half-life of 13.4 h (range 4 – 27.8 h) in nine cases of boric acid poisoning. | The report considered human exposure to equivalent boron doses calculated from compounds such as boric acid, boron oxide, borate salts (e.g. calcium borate) and various hydration states of sodium borate salts (anhydrous, pentahydrate, decahydrate). | ATSDR Report, 2010 |
| ***In vivo* human excretion of boron, specifically examining renal clearance**<br><br>16 pregnant women in the 2nd trimester (14 – 28 weeks) and 15 nonpregnant women (designated as age-matched references).<br><br>Blood samples for boron, creatinine and urea were collected at the start, at 2 h and 24h. Urine was collected during the first 2h | The pregnant and non-pregnant boron intake was 1.35 mg boron/24h and 1.31 mg boron/24h, respectively.<br><br>Renal clearance for 2h period:<br>Renal boron clearance measured over the initial 2h was 68.30 ± 35.0 mL/min/1.73 $m^2$ for pregnant subjects and 54.31 ± 19.35 mL/min/1.73 $m^2$ for non-pregnant subjects based on surface area. Based on body weights, the renal clearances were 1.02 ± 0.55 mL/min/kg and 0.8 ± 0.31 mL/min/kg for pregnant and nonpregnant subjects respectively. | The source of boron used for the measurement of renal boron clearance was the dietary boron normally present in human food (present in high amounts especially in fruits and vegetables). | Pahl et al. 2001 |

| Method | Results | Remarks[3] | Reference |
|---|---|---|---|
| in the Clinical Research Centre and during 22 h outside the centre for measurement of volume, boron and creatinine. | Renal clearance for 24h period<br>The renal clearance was 61.04 ± 36.7 mL/min/1.73 $m^2$ for pregnant subjects and 43.85 ± 21.59 mL/min/1.73 $m^2$ for nonpregnant subjects based on surface area. Based on body weights, the renal clearances were 0.92 ± 0.59 mL/min/kg and 0.64 ± 0.4 mL/min/kg for pregnant and nonpregnant subjects, respectively.<br><br>Plasma levels:<br>The baseline plasma levels of boron were 0.022 ± 0.013 and 0.023 ± 0.015 mg B/mL for nonpregnant and pregnant subjects respectively. At 2h and 24h, the levels were as follows: 2 hours: 0.024 ± 0.015 and 0.018 ± 0.011 mg B/mL for non-pregnant and pregnant subjects respectively; 24 hours: 0.027 ± 0.018 and 0.013 ± 0.006 mg B/mL for non-pregnant and pregnant subjects respectively.<br>Differences in the serum creatinine clearances indicated that urine collection had not been complete over the entire 24 h collection period.<br><br>Comparison of renal boron clearance with creatinine clearance indicated that tubular reabsorption of boron occurred in both pregnant and non-pregnant women. | Purity: unknown<br><br>Reliability: 1 (reliable without restriction). | |
| **Neutron activation analysis-electrothermal atomic absorption spectroscopy (ETA-AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) analysis**<br><br>46 elements from urine, blood and serum of unexposed Italian subjects living in the same region, were determined.<br>The subjects were considered representative of five subgroups resident in urban, suburban, rural and low and high hill areas.<br>A questionnaire supplied detailed information on age, sex, area of residence, occupation, smoking habits, body weight, alimentary habits, socioeconomic and ethnic factors as well as on the elemental composition of the drinking water from | Boron was not present in the blood or serum of healthy Italian subjects.<br><br>Boron was present in the urine of 119 subjects. The mean concentration ± standard deviation was 1890 ± 126 µg/L; with an experimental range of 470 – 7800 µg/L.<br><br>The reference values were 9490 - 3290 µg/L and range of uncertainty was > 3290 – 7800 µg/L.<br><br>The upper limit form metabolic anomalies was > 7800 µg/L. | Environmental exposure to boron<br><br>Reliability: 2 (reliable with restrictions) | Minoia et al. 1990 |

| Method | Results | Remarks[3] | Reference |
|---|---|---|---|
| the municipal supply and mineral water used. | | | |
| *Barium salts* | | | |
| **Inhalative exposure to soluble barium compounds**<br><br>18 workers (welders) exposed for one week to welding fumes containing 31 – 37% barium, were investigated.<br><br>The workers were divided in three groups (A, B, C) based on the type of electrodes used, where the first two groups had no ventilation system at the working site, as opposed to group C. The workers did not use Ba-containing consumables minimum 10 days before the study was performed. On average, the welders worked for about 4 h per day with a mean arc time of about 80 %.<br><br>The investigation included measurements of the external exposure to total welding fumes and soluble Ba in the breathing zone behind the welding shields and helmets, assessment of internal exposure to Ba by biological monitoring of plasma and urine spot samples, medical history taking, thorough clinical and neurological investigations, ECG (limb and precordial leads), continuous 24h ECG (two channels), and measurement of plasma electrolytes (sodium, potassium, magnesium, and total and ionized calcium). Whole blood was checked for pH, standard bicarbonate, and base excess The activities of tubular renal enzymes [N-acetyl P-D-glucosaminidase (NAG) and alanine aminopeptidase (AAP)] | <u>Excretion:</u><br><br>Group A: increased renal excretion of Ba, with median concentrations of 101.7 µg/L urine and 89.1 µg/g creatinine. The highest concentrations in the individual spot samples were 407.7 µg/L urine and 370.6 µg/g creatinine.<br><br>Group B: increased renal excretion of Ba, with median concentrations of 113.1 µg/L urine and 77.3 µg/g creatinine. The maximum individual values were 313.8 µg/L urine and 287.9 µg/g creatinine.<br><br>Group C: slightly increased renal excretion of Ba, with median concentrations of 44.3 µg/L urine and 49.2 µg/g creatinine. The highest individual concentrations were 4.1 µg/L urine and 3.1 µg/g creatinine.<br><br><u>Plasma levels</u>:<br><br>Group A: marked increase in Ba plasma levels, with a median concentration of 24.7 µg/L. The individual post shift concentrations were in the range of 4.1 – 63.4 µg/L.<br><br>Group B: marked increase in Ba plasma levels, with a median concentration of 16.6 µg/L. The individual postshift concentrations were in the range of 4.5 – 74.0 µg/L.<br><br>Group C: slightly increased Ba plasma levels, with a median concentration of 4.4 µg/L. The individual postshift concentrations were in the range of 1.2 – 7.9 µg/L.<br><br>The results show that airborne barium was absorbed either after mucociliary clearance from the gastrointestinal tract or through the respiratory system.<br><br>The biological half-life time of Ba was calculated based on both urine and plasma and found to be 10 – 18h. | Barium-containing stick electrodes or barium-containing self-shielded flux cored wires | Zschiesche et al. 1992 |

| Method | Results | Remarks[3] | Reference |
|---|---|---|---|
| were measured in urine spot samples. | | | |
| **Emission spectrography was used to assess the levels of barium in human tissues**<br><br>A number of approx. 400 subjects (between 0 – 80 years of age) from the United States, Africa, Switzerland, Near Est and Far East were investigated. | Mean plasma concentration of Ba: 79 µg/L<br>Calculated renal plasma clearance of Ba: 330 mL/day<br><br>Urinary excretion of Ba: 3% of the total ingested amount<br><br>Ba was also found in the testis in 75% of the collected samples, and in the ovaries in 87% of the collected samples. The authors also conclude that Ba crosses the placental barrier based upon the fact that Ba is found in infants and children in the first decade of life and even in the stillborn. | The source of Ba used for the measurements performed by this study is represented by dietary Ba normally present in food and water | Schroeder et al. 1972 |
| **Literature review of published and proprietary data** | Absorption: gastrointestinal absorption was 20% in adults, 30% for 1-15 year old children, 60% for infants. Airborne Ba can either be absorbed by the respiratory system or from the gastrointestinal tract (after mucociliary clearence).<br><br>Distribution: 90% was detected in the bone where Ba was primarily deposited in active bone growth areas; 1-2% of the total body burden was found in muscle, adipose, skin and connective tissue.<br><br>Metabolism: barium is not metabolised in the body, but it can be transported or incorporated into complexes or tissues.<br><br>Elimination and excretion: primarily through faecal excretion (approx. 90%) and only 2 – 3% through urine. | The reports considered human exposure to barite and barium salts (e.g. barium chloride, barium nitrate, barium hydroxide, barium sulphate, barium carbonate), which occurred environmentally, occupationally, intentionally or accidentally. | ATSDR report, (2007)<br><br>CICAD WHO report, (2001)<br><br>REACH registration (ECHA dissemination, [2019]) |
| **Animal data** | | | |
| *Boric acid* | | | |
| Rat (Sprague - Dawley), female<br><br>n (renal clearance study) = 10 non-pregnant/group and 10 pregnant/group<br><br>n (half-life study) = 6 non-pregnant/group and 6 pregnant/group<br><br>Exposure: oral (gavage), single administration | Excretion: renal clearance of boron in non-pregnant rats was slightly lower than the renal clearance of boron in pregnant rats (i.e. 3.1 ± 0.8, 3.0 ± 0.6 and 3.2 ± 0.5 mL/min/kg, respectively; and in pregnant rats was 3.3 ± 0.6, 3.2 ± 0.5 and 3.4 ± 0.5 mL/min/kg, respectively).This difference in clearance between pregnant and non-pregnant rats was not statistically significant. The clearance was independent of doses up to 30 mg /kg bw (5.24 mg B/kg bw).<br><br>Half-life: the plasma half-life of boric acid in non-pregnant and pregnant rats given boric | Test material: boric acid<br><br>Purity: > 99%<br><br>Reliability[4]: 1 (reliable without restriction), key study in REACH registration | Vaziri et al. 2001<br><br>REACH registration (ECHA dissemination, [2018]) |

[4] The reliability score for this study is according to the publically disseminated REACH Registration dossier for boric acid, available at https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15472/7/2/2

| Method | Results | Remarks[3] | Reference |
|---|---|---|---|
| Doses/conc.:<br>- Renal clearance study: 0.3, 3.0 or 30 mg boric acid/kg bw equivalent to 0.05, 0.52 and 5.2 mg boron /kg bw, respectively.<br>-Plasma half-life study: 30 mg boric acid/kg, equivalent to 5.24 mg B/kg bw. | acid by gavage was 2.93 ± 0.24 and 3.23 ± 0.28 hours, respectively.<br><br>Identified metabolites: no, boric acid is not metabolised.<br><br>The authors concluded that pregnancy did not induce a statistically significant alteration of the renal clearance or plasma half-life of boron in rats. | | |
| Rat (Fischer 344) male oral: feed<br><br>n = 6/dose group<br><br>Exposure: oral (feed), for 9 weeks<br><br>Doses/conc.: 0, 3000, 4500, 6000 and 9000 ppm boric acid, equivalent to 0, 545, 788, 1050 and 1575 ppm boron (< 0, 0.2, 26, 38, 52, 68 mg B/kg bw/day), respectively. | Distribution: mean (± SD) testis B levels over the 9-week period were 5.6 ± 0.8, 8.8 ±0.7, 11.9 ± 1.4 and 15.1 ± 1.9 µg/g for 3000, 4500, 6000 and 9000 ppm boric acid, respectively.<br><br>Mean (± SD) serum B levels (weeks 1, 4 and 9) were 6.7 ± 1.0, 10.3 ± 0.6, 13.3 ± 0.7 and 17.3 ± 2.2 µg/g for 3000, 4500, 6000 and 9000 ppm boric acid, respectively.<br><br>Identified metabolites: no, boric acid is not metabolised. | Test material: boric acid<br><br>Purity: 99.99%<br><br>Reliability: 2 (reliable with restrictions) | Ku et al. 1993 |
| Rat (Fischer 344), male<br><br>n = 30/group<br><br>Exposure: oral (feed), daily for 7 days<br><br>Doses/conc: 0 and 9000 ppm (1575 ppm boron), equivalent to 0 and 94 mg B/kg bw/day. | Distribution: Plasma and all soft tissues examined, including the testis, epididymis, prostate, seminal vesicles and secretions, hypothalamus,and rest of brain, appeared to reach steady state boron levels (range 12 – 30 µg/g) by 3 – 4 days, with the exception of bone and adipose tissue. Bone boron levels continued to increase up to the termination at 7 days (40 – 50 µg/g by day 7).<br><br>**Boron levels in examined tissues**<br>Control boron levels in plasma and all tissues examined were below 4 µg/g (range 0.66-3.69 µg/g), with the exception of adrenal glands (7.99 µg/g):<br>- Plasma 1.94 ± 0.17;<br>- Liver 0.66 ± 0.10;<br>- Kidney 1.55 ± 0.03;<br>- Adipose tissue 1.71 ± 0.17;<br>- Muscle 3.69 ± 0.54;<br>- Bone 1.17 ± 0.19;<br>- Large intestine 3.08 ± 0.17;<br>- Brain 0.76 ± 0.02;<br>- Hypothalamus 0.91;<br>- Testis 0.97 ± 0.10;<br>- Epididymis 0.81 ± 0.15;<br>- Seminal vesicles 1.64 ± 0.23;<br>- Seminal vesicle fluid 2.05;<br>- Adrenals 7.99;<br>- Prostate 1.20. | Test material : boric acid<br><br>Purity: unknown<br><br>Reliability: 2 (reliable with restrictions) | Ku et al. 1991 |

| Method | Results | Remarks[3] | Reference |
|---|---|---|---|
| | Day 1 (µg B/g tissue, compared to controls):<br>- bone showed a 20-fold increase (i.e. 23.57 ± 1.19);<br>- hypothalamus, rest of brain, liver and kidney showed 12- to 15-fold increases (i.e. 10.90, 11.20 ± 0.47, 10.09 ± 0.60 and 19.53 ± 1.62, respectively);<br>- testis, epididymis, seminal vesicles, seminal vesicle secretions, and prostate showed 7- to 11-fold increases (i.e. 10.41 ±0.78, 8.89 ± 1.10, 14.40 ± 3.87, 14.90 and 13.90, respectively);<br>- plasma, adrenal glands, large intestine and muscle showed only a 2- to 6-fold increase (i.e. 10.82 ± 0.50, 17.40, 10.87 ± 0.72 and 13.73 ± 0.97, respectively).<br><br>All of the soft tissues examined, including the epididymis and accessory sex organs, as well as the testis, hypothalamus, and rest of brain did not show boron accumulation over plasma levels, with a mean tissue/plasma ratio of 1.11 ± 0.05 (mean ± SE) at both days 4 and 7, excluding bone and adipose tissue.<br><br>Days 4 - 7 (compared to controls):<br>- bone showed a 37-fold increase (i.e. 16.37 ± 1.42 - 16.00 ± 0.71);<br>- epididymis, liver, hypothalamus, testis, seminal vesicles and prostate showed 15- to 22-fold increases (19.40 ± 1.46 - 16.81 ±3.7, 12.33 ± 0.37 - 13.13 ± 0.54, 14.80 – 14.30, 14.50±1 .71 - 16.00±1 .19, 27.87 ± 9.80 - 23.70 ± 6.56 and 19.10 – 14.8, respectively);<br>- plasma, kidney and seminal vesicle secretions showed 8- to 13-fold increases (i.e. 16.37 ± 1.42 - 16.00 ± 0.71, 19.77 ±1.60 - 19.80 ± 1.65 and 24.70 – 19.20, respectively);<br>- adrenals, muscle and large intestine, all showed boron concentrations >3 µg/g, (3- to 5-fold increases, i.e. 22.30 - 21.90, 13.20 ± 0.99 - 14.23 ± 0.19 and 16.43±0.94 - 14.90 ± 0.7);<br>- adipose tissue showed a 2-fold increase, i.e. 3.45 ± 0.22 - 3.78 + 0.13.<br><br>Identified metabolites: no, boric acid is not metabolised. | | |
| **Literature review of published and proprietary data** | Absorption: oral absorption fraction in rats was found at 95%. Boron is readily absorbed through damaged skin in rabbits.<br><br>Distribution: in male rats, boron is evenly distributed to liver, kidney, brain, muscle, adrenals, epididymis, testes, seminal vesicles, and blood, but not fat, following 61 | The report considered experimental animal exposure to equivalent boron doses calculated from compounds such | ATSDR report, (2010) |

| Method | Results | Remarks[3] | Reference |
|---|---|---|---|
| | mg boron/kg/day as boric acid for 28 days. In rats, boron accumulates in the bone, reaching 3-fold higher levels than in the soft tissue.<br><br>Metabolism: being an inorganic element, boron is not metabolised by animals, but the parent borate is recovered in the blood, tissues and urine.<br><br>Elimination and excretion: excretion primarily through renal elimination, with a renal clearance value of 163 mg/kg/ hour in rats. | as boric acid, boron oxide, borate salts (e.g. calcium borate) and various hydration states of sodium borate salts (anhydrous, pentahydrate, decahydrate), which occurred through various routes of exposure (i.e. inhalation, oral, dermal, intravenous and intra-tympanic). | |
| *Barium salts* | | | |
| Rat (brown-hooded August), female<br><br>n = 10/group for age groups 14-18 days and 60 – 70 weeks, and 5/group for age group 6 – 8 weeks of age<br><br>Exposure: oral (gavage), single administration<br><br>Doses/conc.: the activity administered as a single dose to each animal was approx. 10 µC of $^{140}$Ba, 1 µC of $^{85}$Sr, 1 µC of $^{45}$SCa, 0.01 µC of $^{226}$Ra.<br><br>Three administration groups were used: fed rats, starved rats and fed rats which also received cow milk administration. Three age groups were used: 14 – 18 days old, 6 - 8 and 60 – 70 weeks old female rats. The animals were sacrificed 7h after administration. | Absorption:<br>- At 14 – 18 days of age, approx. 80% of Ba was absorbed;<br>- For 6 – 8 weeks of age, the absorption of Ba decreased to approx. 7%;<br>- For 60 – 70 weeks of age, the absorption of Ba was approx. 7.5%.<br><br>The absorption of Ba was markedly increased by food deprivation before exposure:<br>- At 6 – 8 weeks of age, approx. 20% of Ba was absorbed;<br>- At 60 – 70 weeks of age, approx. 19% of Ba was absorbed.<br><br>The administration of cow milk had no effect on the absorption of Ba. | Test material: Barium chloride, strontium chloride, radium chloride, calcium chloride<br><br>Purity: unknown | Taylor et al. 1962 |
| **Literature review of published and proprietary data** | Absorption: rapid absorption following inhalation or nasal deposition with more efficient clearance in the upper respiratory tract than in the trachea (0.41, 0.145, 0.044, and 0.043% retained $^{133}$Ba in the trachea one week after administration for rats, rabbits, dogs, and monkeys, respectively).<br><br>Gastrointestinal absorption was approx. 50% in dogs, compared to 30% in rats and mice. | The reports considered experimental animal exposure to barium salts (e.g. barium chloride, barium barium sulphate, barium carbonate), | REACH registration (ECHA dissemination, [2019])<br><br>ATSDR report, (2007) |

| Method | Results | Remarks[3] | Reference |
|---|---|---|---|
| | Younger rats absorbed approx. 10 times more (i.e. 63-84%) barium from the gastrointestinal tract than older rats (approx. 7%).<br><br>Distribution: predominantly in the bone, with the following non-skeletal distribution 24h after ingestion in rats: heart > eye > skeletal muscle > kidney > blood > liver.<br><br>Metabolism: Ba is not metabolised in the body, but it can be transported or incorporated into complexes or tissues.<br><br>Elimination and excretion: faecal excretion exceeds urinary excretion in the case of rats, dogs and rabbits.<br><br>A biological half-life time of 12.8 days following inhalation exposure, was estimated in dogs. | which occurred through various routes of exposure (i.e. inhalation, oral, dermal and intravenous). | CICAD WHO report, (2001) |
| **Comparative review of the toxicokinetics of boric acid in humans and animals** | | | |
| **Literature review of published data** | Absorption:<br>- Oral absorption: humans and animals (rats, rabbits, sheep and cattle) absorb boric acid similarly, i.e. readily and completely from the gastrointestinal tract.<br>- Dermal absorption: negligible absorption across intact skin for both animals and humans; for non-intact skin, the absorption varies with the used vehicle.<br><br>Distribution: similar distribution of boric acid in both animals and humans, i.e. through the body fluids, with boron not accumulating in the soft tissue:<br>- For humans, boron levels found in soft tissues were equivalent to those found in plasma, while boron levels found in bone were higher than those in soft tissues or plasma. High levels of boron were also found in hair and teeth.<br>- Similar to humans, the highest levels of boron for rats and mice was found in the bone, reaching 2-3 times those observed in plasma, and continued to increase throughout 7 days of exposure. However, the boron levels found in adipose tissue represented only 20% of the plasma ones. The levels of boron measured in the testis of male rats were almost equivalent to those measured in plasma.<br><br>Metabolism: boric acid is not metabolised in either humans or animals. Other borate salts convert to boric acid at physiological pH in the aqueous layers of the mucosal surfaces. | The review considered both human and experimental animal exposure to boric acid, which occurred through various routes of exposure (i.e. oral, dermal, intravenous). | Murray 1998 |

| Method | Results | Remarks[3] | Reference |
|---|---|---|---|
| | Excretion and elimination: irrespective of the route of exposure, boric acid is excreted unchanged through the urine, in both humans and animals, with a half-life of < 24h, and it can be slowly eliminated from bone.<br><br>Blood levels: in male rats, a close degree of correlation between plasma levels and testicular levels was found, and thus a testes level of 5.6 µg B/g (corresponding to 26 mg B/kg bw/day) was associated with mildly inhibited spermiation while testicular atrophy was observed at a concentration of 11.9 µg B/g (equivalent to 52 mg B/kg bw/day). | | |

## 9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

There is no available *in vivo* information on the toxicokinetic properties of barium diboron tetraoxide and there is limited toxicity data available for the hazard class (reproductive toxicity) considered in this CLH proposal. Classification for reproductive toxicity following oral exposure is therefore supported using a read-across approach from tested borates (borax or disodium tetraborate decahydrate) and boric acid, justified on the basis of hydrolytic and toxicokinetic behaviour, and toxicological data (see section 9.1.1 below for read-across justification). Moreover, data on barium, as the counter ion, are also considered in the hazard assessment of barium diboron tetraoxide since its contribution to potential adverse effects cannot be dismissed.

### 9.1.1 Justification for read-across from boric acid and borate salts

Barium diboron tetraoxide is the inorganic ionic salt of boric acid. Barium diboron tetraoxide is described as slightly to moderately soluble in water, and soluble in hydrochloric acid. The solubility of barium diboron tetraoxide in water is 822 mg/L at 25°C, which is expected to increase at gastric pH and physiological temperature. Based on the chemical nature of the substance, it is predicted to dissociate into its constituent ions, $Ba^{2+}$ ion and the metaborate ion ($BO_2^-$), under physiological conditions and prior to absorption.

Following administration and prior to absorption into the systemic circulation, barium diboron tetraoxide will dissociate in body fluids, as for example saliva, the aqueous layer overlaying the mucosal surfaces and gastric fluid during oral administration. Therefore, aqueous solutions of this borate contain only boric acid $H_3BO_3$, its conjugate base $B(OH)_4^-$ and the counter ion ($Ba^{2+}$). The relative concentrations of the boron species is a function of pH. Boric acid is the main species at acidic and neutral pH. At an alkaline pH (above pH 10) the metaborate anion $B(OH)^-_4$ becomes the main species in solution. More concentrated borate solutions also contain at the intermediate pH range polyborate anions ($B_5O_6(OH)_4^-$, $B_3O_3(OH)_4^-$, $B_4O_5(OH)_4^{2-}$ and $B_3O_3(OH)_5^{2-}$). The distribution of species is largely independent of the cation.

From the species distribution of borates, it can be concluded that the main borate species at physiologically relevant conditions (large volume of distribution, aqueous solution, acidic or neutral pH) is boric acid. In addition, as stated in the report on boron performed in 1998 by the International Programme on Chemical Safety (IPCS)[5], studies performed with rats, rabbits, sheep and cattle

[5] http://www.inchem.org/documents/ehc/ehc/ehc204.htm#PartNumber:6

indicated that more than 90% of administered doses of inorganic borates were excreted in the urine as boric acid. The systemic effects of borates are therefore considered to be related to the concentration of boric acid systematically available. Since the oral bioavailability of boric acid is nearly 100 %, it is assumed that the transport of boric acid across the intestinal wall only depends on the concentration of boric acid in the intestine. The intestinal concentration depends on the administered dose and the solubility and dissolution rate of the specific borate in gastric fluid.

Additionally, as also stated in the IPCS report on boron, the chemical and toxicological effects of boric acid and other borates are similar on a mol boron/liter equivalent basis when dissolved in water or biological fluids at the same pH and low concentration. Therefore, read-across to boric acid and borate salts for both toxicokinetic properties and systemic effects, based on boron (B) equivalents is justified.

These equivalents were obtained through using the following values (derived based on the molar masses of barium diboron tetraoxide, boric acid, borax and boron) and conversion factors for administration of the test substances in drinking water or feed (EFSA, 2012):

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

As stated in the CLH-reports of disodium octaborate, anhydrate and disodium octaborate tetrahydrate (2013) read-across from boric acid to other borates and between borates has long been accepted in a regulatory context. Experts from the CL Working Group, the TC-C&L and the ATP Committee agreed that borates have similar properties and therefore that read-across between substances can be applied.

#### 9.1.1.1 Consideration of barium as the counter ion

As stated above, under physiologically relevant conditions and prior to absorption, barium diboron tetraoxide is expected to dissociate into the metaborate and barium ions. Since barium can be distributed to both the testes and ovaries and can cross the placental barrier (Schroeder et al. 1972), its potential contribution to the overall reproductive toxicity of barium diboron tetraoxide is also assessed in the current CLH proposal.

As shown by the IPCS 1991 report[6], 20% of an ingested dose of a barium compound of an unspecified solubility was found after 24h primarily in the faeces (20%), but also in urine (5 – 7%). Furthermore, the rate of absorption for barium is dependent on the route of exposure and age. The human data show that the gastrointestinal absorption for barium was approx. 60% in infants as opposed to 20% in adults, while the animal data show that younger rats absorbed approx. 10 times more (i.e. 63 – 84%) barium than older ones (i.e. 7%).

Therefore, it appears that barium has a lower absorption rate than boric acid and borate salts, and a different elimination route (90% through faeces for barium vs. 90% through renal elimination for boric acid).

### 9.1.2 Toxicokinetic data on boric acid and borate salts

No studies according to validated test guidelines on the toxicokinetics of boric acid or borate salts are available. The data described above in Table 7 are mainly represented by what is available in the open scientific literature as experimental (animal data) and occupational studies, and literature reviews.

***Absorption***
Oral

[6] http://www.inchem.org/documents/ehc/ehc/ehc107.htm#SectionNumber:6.3

Humans and animals (rats, rabbits, sheep and cattle) absorb orally administered boric acid in a similar way, readily and completely from the gastrointestinal tract, with 92 – 95% of the dose being recovered in the urine.

Inhalation
After boric acid exposure via inhalation, boron is systemically distributed through absorption across pulmonary tissues and into the bloodstream.

Dermal
The available studies show that there is minimal dermal absorption (i.e. 0.5%) of boric acid through intact skin for both animals and humans. Absorption through non-intact skin varies with the used vehicle: as opposed to oil-based vehicle, aqueous-based ones lead to a greater dermal absorption of boric acid.

***Distribution***
After administration of boric acid, boron has a similar distribution for both humans and animals with the following common aspects:

- Boron is rapidly distributed throughout body fluids;
- Boron does not accumulate in soft tissue;
- Boron accumulates in the bone, reaching 2 – 3 times higher levels than in plasma.

The plasma and soft tissue concentrations of boron are equivalent for humans, while the adipose tissue levels of boron represented only 20% of the plasma ones in rats. The testis levels of boron in male rats were almost equal to the ones measured in plasma. Moreover, in male rats, a close correlation between testicular and blood levels of boron was found, with testicular concentrations of 5.6 µg B/g (equivalent to 26 mg B/kg bw/ day) and 11.9 µg B/g (equivalent to 52 mg B/kg bw/ day) being associated with inhibited spermiation and testicular atrophy, respectively (Murray et al. 1998).

***Metabolism***
Boric acid is not metabolised in either humans or animals, boron being a trace element which exists in the body as boric acid (the only form of boron recovered in the urine).

***Excretion and elimination***
Independently of the route of exposure, boric acid is primarily excreted through renal elimination and has a half-life less than 24h for both humans and animals. It can also be slowly eliminated from the bone. Based on literature data, eliminated fractions of absorbed boron were estimated to be 67 – 98% for humans and 99% for rats (ATSDR 2010), and the calculated clearance values were 40 mg/kg/hour in humans and 163 mg/kg/hour in rats, respectively. In addition, the glomerular filtration rate appears to be the determining factor in the renal elimination of boron.

### 9.1.3 Toxicokinetic data on barium salts

***Absorption***
Oral
Oral absorption of barium decreases with age for humans (20% for adults vs. 60% for infants) and animals (80 % for rats at 2 – 3 weeks of age vs. 7.5% for rats at 60 – 70 weeks of age) (ATSDR 2007). Moreover, the oral absorption of barium appears to be facilitated by food deprivation before exposure (approx. 20% for starved rats of 6 – 8 weeks of age vs. approx. 7% for fed rats of 14 – 18 days of age), as shown by Taylor et al. 1962.

Inhalation
Barium is rapidly absorbed following inhalation exposure, with intratracheal retention.

Dermal

Due to the high polarity of forms in which barium is usually available, it is not expected to cross the intact skin.

***Distribution***

Similarly, to boron, approx. 90% of the absorbed barium accumulates in the bone where it is primarily deposited in active growth areas, in both humans and animals. Following 24h after ingestion, the following non-skeletal distribution in rats was observed: heart > eye > skeletal muscle > kidney > blood > liver, while in humans 1-2% of the total body burden was found in the muscle, adipose, skin and connective tissue. Additionally, human data show that Ba can also be distributed to the testis and ovaries. It also has the ability to cross the placental barrier as demonstrated by the fact that barium was found in infants and children in the first decade of life and even in the stillborn (Schroeder et al. 1972).

***Metabolism***

Barium is not metabolised in the body but it can be transported or incorporated into complexes or tissues.

***Excretion and elimination***

For both humans and animals (i.e. rats, rabbits and dogs), barium is primarily excreted through faeces (approx. 90%), while only approx. 3% accounts for urinary excretion.

# 10 EVALUATION OF HEALTH HAZARDS

## Acute toxicity

## 10.1 Acute toxicity - oral route

### Table 8: Summary table of animal studies on acute oral toxicity

| **Method, guideline, deviations if any**[7] | **Species, strain, sex, no/group** | **Test substance** | **Dose levels, duration of exposure** | **Value $LD_{50}$** | **Reference** |
|---|---|---|---|---|---|
| *Barium diboron tetraoxide* | | | | | |
| **Non-guideline acute oral toxicity study**<br><br>Similar to OECD 401. Conducted prior to GLP, and the availability of OECD guidelines. No information on the purity of the test sample, limited information on the animals and the testing | Rat (Sprague-Dawley)<br><br>male/female<br><br>n = 8/sex/group | Busan 11-M1 (barium metaborate monohydrate)<br><br>Purity: unknown<br><br>Vehicle: unknown<br><br>Oral gavage | 340, 500, 730, 1070, 2310 and 5000 mg/kg bw<br><br>Single oral dose<br><br>14 days post-exposure observation period | Males: 850 mg/kg bw<br><br>Females: 530 mg/kg bw | REACH registration (ECHA dissemination, [2019])<br><br>Study report 1979a |

[7] The reliability score for this study is according to the publically disseminated REACH Registration dossier for barium diboron tetraoxide, available at https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15812/7/3/2

| Method, guideline, deviations if any[7] | Species, strain, sex, no/group | Test substance | Dose levels, duration of exposure | Value $LD_{50}$ | Reference |
|---|---|---|---|---|---|
| conditions (such as on temperature and humidity).<br><br>Reliability: 2 (reliable with restrictions) | | | | | |

**Table 9: Summary table of human data on acute oral toxicity**

| Type of study/data | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No human data were available on the acute oral toxicity of barium diboron tetraoxide | | | | |

**Table 10: Summary table of other studies relevant for acute oral toxicity**

| Type of study/data | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No other relevant studies on the acute oral toxicity of barium diboron tetraoxide were available | | | | |

### 10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

#### Animal studies

Barium diboron tetraoxide

One non-guideline study (preceding OECD test guidelines and GLP) of acceptable quality and reliability investigating the acute oral toxicity of barium diboron tetraoxide in rats was available. An $LD_{50}$ of 850 mg/kg bw was established for males and 530 mg/kg bw for females.

Since this study provides sufficient substance-specific information on barium diboron tetraoxide for classification, no read-across from data on boric acid and borate salts or barium salts is performed. The below data on boric acid, borate salts and barium salts are presented only for comparison.

Boric acid and borate salts

According to the disseminated REACH registration dossier[8], based on a non-guideline study performed in rats, an $LD_{50}$ of 3450 mg/kg bw was established for boric acid. Other non-guideline acute oral toxicity studies in rats also reported $LD_{50}$ > 2000 mg/kg bw.

Moreover, acute oral toxicity studies performed in rats for disodium octaborate anhydrate, disodium tetraborate anhydrous, disodium tetraborate pentahydrate and diboron trioxide revealed $LD_{50}$ levels of > 2000 mg/kg bw, for each substance.

Barium chloride

According to the disseminated REACH registration dossier of barium chloride[9], the acute oral toxicity study performed in rats according to OECD TG 401 established an $LD_{50}$ of 645 mg/kg bw (equivalent to an $LD_{50}$ level of 426 mg Ba/kg bw). However, two additional non-guideline studies revealed lower

[8] https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15472/7/3/1

[9] https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15037/7/3/2

$LD_{50}$ levels for barium chloride (i.e. $LD_{50} \leq 300$ mg/kg bw). The reported acute oral toxic effects in rats mainly consist of increased or decreased respiratory rate and irregular respiration, increased or decreased motility, loss of coordination, paralysis of the hind legs, tremors and coarse body tremors.

**Human data**

Barium diboron tetraoxide

No human data on the acute toxicity of barium diboron tetraoxide were available.

Boric acid and borate salts

As detailed in the disseminated REACH registration dossier of boric acid[10], intentional or accidental poisoning incidents with boric acid or borate salts have been reported. Based on an old case review study, the human oral lethal dose was reported as 2-3 g boric acid for infants, 5-6 g boric acid for children and 15-30 g boric acid for adults. None of the more recent poisoning cases with an estimated dose range of 0.01 – 88.8 g boric acid were reported to be fatal. The reported acute effects are mainly represented by nausea, vomiting, gastric effects, skin flushing, convulsions, depression and vascular collapse.

Barium salts

Death has been reported in several cases of accidental or intentional ingestion of barium salts, primarily caused by cardiac arrest and severe gastrointestinal haemorrhage (Deng et al. 1991; Diengott et al. 1964; Downs et al. 1995; Jourdan et al. 2001). However, the doses in these cases were not known.

***Conclusion***

The available acute oral toxicity study of barium diboron tetraoxide meets the criteria for classification in Acute Tox. 4, and the lowest $LD_{50}$ is 530 mg/kg bw in the rat (females). In comparison, the $LD_{50}$ values for boric acid and borate salts (i.e. disodium octaborate anhydrate, disodium tetraborate anhydrous, disodium tetraborate pentahydrate and diboron trioxide) were reported to be > 2000 mg/kg bw, and thus, not requiring classification. However, the higher acute oral toxicity of barium diboron tetraoxide could be due to the barium counter ion, for which an $LD_{50}$ value of 426 mg/kg bw (corresponding to Category 4) can be derived from the $LD_{50}$ of 645 mg/kg bw for barium chloride (also corresponding to Category 4). Thus, correcting for the percentage of barium, the $LD_{50}$ established for barium diboron tetraoxide (i.e. 530 mg/kg bw) would correspond to 302 mg Ba/kg bw (Category 4).

### 10.1.2 Comparison with the CLP criteria

According to the CLP Regulation (EC) No 1272/2008, classification for acute oral toxicity is required for substances with acute toxicity estimate values (based on $LD_{50}$) below 2000 mg/kg bw. Category 3 is assigned for ATE values > 50 and ≤ 300 mg/kg bw, while Category 4 is assigned for substances with ATE values > 300 and ≤ 2000 mg/kg bw.

The acute oral toxicity study performed in rats with barium diboron tetraoxide established $LD_{50}$ values > 300 and < 2000 mg/kg bw (Category 4), where the lowest $LD_{50}$ was 530 mg/kg bw.

### 10.1.3 Conclusion on classification and labelling for acute oral toxicity

Classification in Acute Tox. **4**, **H302**, with an ATE of 530 mg/kg bw, is proposed for barium diboron tetraoxide.

Currently, barium diboron tetraoxide has a harmonised classification as Acute Tox. 4* (H302) for the oral route of exposure, as part of a group entry in Annex VI of CLP. A removal of the asterisk (*) indicating minimum classification is thus proposed.

[10] https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15472/7/11/1

## 10.2 Acute toxicity - dermal route

### Table 11: Summary table of animal studies on acute dermal toxicity

| Method, guideline, deviations if any[11] | Species, strain, sex, no/group | Test substance, | Dose levels duration of exposure | Value $LD_{50}$ | Reference |
|---|---|---|---|---|---|
| *Barium diboron traoxide* | | | | | |
| **Non-guideline acute dermal toxicity study**<br><br>Similar to OECD 402. No information on the purity of the test substance, limited information on the animals and the testing conditions (such as on temperature and humidity), no information on the size of test site. The study was conducted on abraded skin and the animals were immobilised.<br><br>Reliability: 2 (reliable with restrictions) | Rabbit (New Zealand White)<br><br>male/female<br><br>n = 5/sex/group | Busan 11-M1 (barium metaborate monohydrate)<br><br>Purity: unknown | 2000 mg/kg bw<br><br>Single dermal dose<br><br>14 days post-exposure observation period | Male/female: > 2000 mg/kg bw | REACH registration (ECHA dissemination, [2019])<br><br>Study report 1979b |

### Table 12: Summary table of human data on acute dermal toxicity

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No human data were available on the acute dermal toxicity of barium diboron tetraoxide | | | | |

### Table 13: Summary table of other studies relevant for acute dermal toxicity

| Type of study/data | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No other relevant studies on the acute dermal toxicity of barium diboron tetraoxide | | | | |

[11] The reliability score for this study is according to the publically disseminated REACH Registration dossier for barium diboron tetraoxide, available at https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15812/7/3/4

### 10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

#### Animal studies

Barium diboron tetraoxide

One non-guideline study (preceding OECD test guidelines and GLP) of acceptable quality and reliability investigating the acute dermal toxicity of barium diboron tetraoxide in rabbits was available. An $LD_{50}$ of > 2000 mg/kg bw was established.

Since this study provides sufficient substance-specific information on barium diboron tetraoxide for classification, no read-across from data on boric acid and borate salts or barium salts is performed. The below data on boric acid, borate salts and barium salts are presented only for comparison.

Boric acid and borate salts

According to the disseminated REACH registration dossier[12] of boric acid, an $LD_{50}$ > 2000 mg/kg bw was established based on a non-guideline study performed in rabbits. Moreover, acute dermal toxicity studies performed in rats or rabbits for disodium octaboarte anhydrate, disodium tetraborate decahydrate and disodium tetraborate pentahydrate revealed $LD_{50}$ levels of > 2000 mg/kg bw, for each substance.

Barium chloride

The acute dermal toxicity study performed in rats according to OECD TG 402 established an $LD_{50}$ > 2000 mg/kg bw. However, it should be noted that the study report was not available for assessment.

#### Human data

Barium diboron tetraoxide

No human data on the acute dermal of barium diboron tetraoxide were available.

Boric acid and borate salts

As detailed in the disseminated REACH registration dossier of boric acid, several poisoning cases were reported in humans due to the use of skin and mucosa antiseptic pharmaceutical preparations containing boric acid. Moreover, case reports of accidental exposure of the head were also reported, with effects such as general or focal alopecia of the scalp (ATSDR Report, 2010).

Barium salts

Limited data are available describing the acute dermal toxicity of barium salts in humans. Due to the high polarity of the forms in which barium is mostly encountered, it is not expected to cross the intact skin. A case report of dermal burns of a worker exposed to molten barium chloride was available (ATSDR Report, 2007). These effects could have however been due to the molten nature of the material and not necessarily due to barium chloride.

#### *Conclusion*

The available data indicate that barium diboron tetraoxide displays low acute dermal toxicity ($LD_{50}$ > 2000 mg/kg bw). The reported information is comparable for boric acid, borate and barium salts, where the established $LD_{50}$ values were > 2000 mg/kg bw, and thus do not require classification.

### 10.2.2 Comparison with the CLP criteria

According to the CLP Regulation (EC) No 1272/2008, classification for acute dermal toxicity is required for substances with acute toxicity estimate values (based on $LD_{50}$) below 2000 mg/kg bw. The reported $LD_{50}$ value of barium diboron tetraoxide indicates no requirement for classification for acute dermal toxicity.

[12] https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15472/7/3/4

### 10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the experimental animal data revealing an $LD_{50}$ value over 2000 mg/kg bw, classification of barium diboron tetraoxide for acute dermal effects is not warranted.

## 10.3 Acute toxicity - inhalation route

**Table 14: Summary table of animal studies on acute inhalation toxicity**

| **Method, guideline, deviations if any[13]** | **Species, strain, sex, no/group** | **Test substance, form and particle size (MMAD)** | **Dose levels, duration of exposure** | **Value** $LC_{50}$ | **Reference** |
|---|---|---|---|---|---|
| *Barium diboron tetraoxide* | | | | | |
| **Non-guideline acute inhalation toxicity study**<br><br>Similar to OECD 403. Conducted prior to GLP, no information on the purity of the test sample, or on the conditions of exposure. The study was carried out at the maximum attainable concentration.<br><br>Reliability: 2 (reliable with restrictions) | Rat (Sprague-Dawley)<br><br>male/female<br><br>n = 5/sex/group | Busan 11-M1 (barium metaborate monohydrate)<br><br>Purity: unknown<br><br>Vehicle: unknown<br><br>Inhalation (whole body): dust<br><br>MMAD (µm): 3.4±0.28 and 2.8±0.14 for 2.98 mg/L and 3.54 mg/L, respectively | Nominal concentration: 14.52 mg/L and 21.70 mg/L<br><br>Mean gravimetric concentration: 2.98 mg/L and 3.54 mg/L<br><br>Exposure duration: 4 h<br><br>14 days post-exposure observation period | Male/female: > 3.5 mg/L | REACH registration (ECHA dissemination, [2019])<br><br>Study report 1983 |

**Table 15: Summary table of human data on acute inhalation toxicity**

| **Type of data/report** | **Test substance,** | **Relevant information about the study (as applicable)** | **Observations** | **Reference** |
|---|---|---|---|---|
| No human data were available on the acute inhalation toxicity of barium diboron tetraoxide | | | | |

**Table 16: Summary table of other studies relevant for acute inhalation toxicity**

| **Type of study/data** | **Test substance,** | **Relevant information about the study (as applicable)** | **Observations** | **Reference** |
|---|---|---|---|---|
| No other relevant studies on the acute inhalation toxicity of barium diboron tetraoxide were available | | | | |

[13] The reliability score for this study is according to the publically disseminated REACH Registration dossier for barium diboron tetraoxide, available at https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15812/7/3/3

### 10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

#### Animal studies

Barium diboron tetraoxide

One non-guideline study (preceding OECD test guidelines and GLP) of acceptable quality and reliability investigating the acute oral inhalation toxicity of barium diboron tetraoxide in rats was available. The test animals were exposed to nominal concentrations of 14.52 mg/L and 21.70 mg/L, corresponding to gravimetric concentrations of 2.98 mg/L and 3.54 mg/L, for 4 hours via whole-body exposure of diboron tetraoxide dust with MMAD within the respirable range (1-4 μm). The highest dose tested was the maximum attainable concentration of barium diboron tetraoxide. After exposure, all animals in the two treated groups appeared languid from 30 min through 4 hours. Some animals at the lower dose levels also showed slight dyspnoea from hour 2 through and rhinorrhoea from hour 1 through hour 4. One male was found dead at 2.98 mg/L at day 2 after exposure and one female rat died at 3.5 mg/L at day 1 after exposure. On day 1 post exposure individuals of the high dose 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. No $LC_{50}$ could be set (> 3.5 mg/L) based on the findings in the study.

Since this study provides sufficient substance-specific information on barium diboron tetraoxide for classification, no read-across from data on boric acid and borate salts or barium salts is performed. The below data on boric acid, borate salts and barium salts are presented only for comparison.

Boric acid and borate salts

According to the disseminated REACH registration dossier[14], based on an OECD 403 study performed in rats, an $LC_{50}$ > 2.03 mg/L was established. In addition, an US EPA FIFRA study performed in rats, reported an $LC_{50}$ of > 2.12 mg/L.

Moreover, acute inhalation toxicity studies performed in rats for disodium octaborate tetrahydrate and disodium tetraborate pentahydrate revealed $LC_{50}$ levels > 2 mg/L, for each substance.

Barium chloride

As reported in the REACH registration dossier of barium chloride[15], an acute inhalation toxicity study performed in rats according to OECD TG 403 established an $LC_{50}$ > 1.1 mg/L (1/5 male rats was found dead 3h after exposure and no other mortalities occurred).

#### Human data

Barium diboron tetraoxide

No human data on the acute toxicity of barium diboron tetraoxide were available.

Boric acid and borate salts

Healthy volunteers were exposed to 0, 5, 10, 20, 30 and 40 mg/m$^3$ sodium tetraborate pentahydrate as dust for 20 min, while cycling (Cain et al. 2004). Effects such as nasal and throat irritation were seen ≥ 30 mg/m$^3$, the subjects reporting time-dependent feel due to sodium tetraborate pentahydrate exposure primarily in the nose and hardly in the eyes. Similarly, healthy volunteers were exposed to 0, 10 mg/m$^3$ sodium borate, and to 0, 2.5, 5 and 10 mg boric acid/m$^3$ for 47 minutes while exercising (Cain et al. 2008). Increased nasal secretions and decreased nasal airway resistance was observed at 10 mg/m$^3$ sodium borate.

Barium salts

[14] https://www.echa.europa.eu/web/guest/registration-dossier/-/registered dossier/15472/7/3/3/?documentUUID=371b867a-f26c-412a-996f-007ef1835888

[15] https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15037/7/3/3

A case report of an accidental exposure to a large but unknown amount of barium carbonate powder of a 22-year old worker was available (Shankle and Keane 1988). Effects such as progressive muscle weakness, paralysis of the extremities and neck, renal failure, abdominal cramps, nausea and vomiting were reported.

***Conclusion***

While the acute inhalation study performed in rats could not establish an $LC_{50}$, the available data indicate that barium diboron tetraoxide displays low acute inhalation toxicity since only 1/5 male rats was found dead at the maximum attainable concentration (i.e. 3.5 mg/L) after 4 hours of exposure. Furthermore, the reported information indicates that boric acid, disodium octaborate tetrahydrate, disodium tetraborate pentahydrate also have low acute inhalation toxicity since no deaths were observed at concentrations > 2 mg/L. In contrast, the data show that barium chloride has a higher acute inhalation toxicity than the borate salts, since one death occurred at 1.1 mg/L.

Based upon the presented data, it is assumed that the $LC_{50}$/ATE is greater than the maximum attainable concentration, and thus, barium diboron tetraoxide does not require classification for acute inhalation toxicity.

### 10.3.2 Comparison with the CLP criteria

According to the CLP Regulation (EC) No 1272/2008, classification for acute inhalation toxicity is required for substances with acute toxicity estimate values (based on $LC_{50}$) below 5 mg/L (dusts and mists). Since the $LC_{50}$ value for barium diboron tetraoxide is greater than the maximum attainable concentration (3.5 mg/L), classification for acute inhalation toxicity is not required.

### 10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the experimental animal data revealing an $LC_{50}$ value over the maximum attainable concentration, classification of barium diboron tetraoxide for acute inhalation toxicity is not warranted.

Currently, barium diboron tetraoxide has a harmonised classification as Acute Tox. 4* (H332) for the inhalation route of exposure, under the group entry entitled “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 EC No 1272/2008”.

Based upon the available data on the acute inhalation toxicity of barium diboron tetraoxide, a removal of the classification as Acute Tox. 4* (H332) is proposed.

## 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.

## Additional key elements

### *Mortality in range-finding studies to the rabbit PNDT study with barium metaborate*

Two range-finding studies via gavage were conducted prior to the main rabbit PNDT study (Study report, 1993b): a 5-day study in non-pregnant females and a limited PNDT study. The substance (Busan 11-M1, purity 94.3%) was administered via gavage in 0.5% aqueous methyl cellulose to female New Zealand White rabbits on days 0-5 in the 5-day study and on gestation days (GD) 7-19 in the PNDT study. Scheduled sacrifice took place on day 5 in the 5-day study and on GD 29 in the PNDT study. Both range-finding studies reported mortalities after a single dose as shown in the tables below.

| **Mortality in the 5-day tolerability study in non-pregnant female rabbits** | | | |
|---|---|---|---|
| **Dose (mg/kg bw/d)** | **100** | **200** | **400** |
| Females in study | 2 | 2 | 2 |
| Found dead | 0 | 2 | 1 |
| Euthanised in extremis | 0 | 0 | 1 |
| Day of death | | 0, 0 | 0, 0 |

| **Maternal mortality in the range-finding rabbit PNDT study** (dosing GD 7-19) | | | | | | |
|---|---|---|---|---|---|---|
| **Dose (mg/kg bw/d)** | **0** | **20** | **55** | **90** | **125** | **160** |
| Females in study | 7 | 7 | 7 | 7 | 7 | 6[a] |
| Found dead | 0 | 1 | 4 | 5 | 7 | 6 |
| Euthanised in extremis | 0 | 1 | 0 | 0 | 0 | 0 |
| Day of death (GD) | | 20, 23 | 9, 14, 15, 16 | 8, 9, 10, 10, 16 | 7, 7, 8, 9, 9, 10, 10 | 7, 7, 7, 7, 8, 8 |

[a] 1 of the 7 dams of the group was still alive on GD 8 but was removed from the study on GD 8 due to the mortality in this group

### *Human intoxications after exposure to soluble barium compounds*

Ingested barium compounds that dissolve in the gastrointestinal tract are readily absorbed (ATSDR, 2007). With an indicated water solubility of 0.8 g/L (CLH report) barium metaborate belongs to the group of soluble barium salts.

The barium ion and the soluble compounds of barium (notably chloride, nitrate, and hydroxide) are generally highly toxic to humans and experimental animals. The insoluble

barium compounds (notably sulfate) are inefficient sources of the barium ion and therefore are generally non-toxic. Although barium carbonate is practically insoluble in water, barium ions would be released from ingested barium carbonate in the acid milieu of the stomach (ATSDR, 2007).

Acute intoxication with soluble barium compounds in humans may cause rapid onset of gastrointestinal symptoms (nausea, salivation, vomiting, abdominal cramps, watery diarrhea) and hypokalemia, a reduction in blood potassium levels that can result in ventricular tachycardia, hypertension and/or hypotension, muscle weakness and paralysis (ATSDR, 2007; WHO, 2001).

Barium also interferes with calcium dependent processes. Like calcium, barium leads to the release of neurotransmitters, but in contrast to calcium this requires no depolarisation of the respective neuron. Consequently, barium results in a muscular stimulation in the beginning and later leads to muscle paralysis (MAK, 2001; WHO, 1990).

Nephrotoxicity is also observed after exposure to barium compounds. While this is sometimes considered a repeated dose effect, two cases of acute oral intoxications resulted in acute renal failure requiring dialysis (NTP, 1994; Fogliani *et al.*, 1993, referenced in MAK, 2010). The NTP study (1994) concludes that acute renal failure upon barium exposure results from a disturbed electrolyte balance, with potassium being most relevant here.

There is little quantitative information regarding the extent of barium absorption following inhalation, oral, or dermal exposure. Available evidence indicates that barium is absorbed to some extent following inhalation, oral, and dermal exposure; however, in some cases, absorption is expected to be limited. For example, there is some evidence that gastrointestinal absorption of barium in humans is < 5–30% of the administered dose (ATSDR, 2007). Based on data from Syrian hamsters, WHO (1990) and MAK (2010) concluded that inhalation uptake might exceed oral uptake. However, no respective data are available in humans. Barium uptake is age-dependent (demonstrated in rats, assumed for humans, MAK, 2010; WHO, 2001) and also depends on other factors like e.g. presence of sulfate in the food or amount of food consumed. Toxicity depends on various factors including pre-existing morbidities (e.g. high blood pressure or electrolyte imbalance, ATSDR, 2007).

In a US CDC Report (2003), it is stated that severe life-threatening intoxication can occur after ingestion or inhalation of "even minute amounts" of the absorbable salts of barium (e.g. barium chloride, carbonate, or sulfide) during radiologic examination (if the insoluble barium sulfate contains contaminations with soluble barium salts) or in occupational settings (e.g. mining, refining operations, production of fireworks or rodenticides). In contrast, ATSDR (2007) indicates that ingestion of "relatively large amounts" leads to severe acute effects and that limited human and animal data indicate that high-level inhalation exposure to soluble barium compounds may result in systemic effects similar to those elicited from high-level oral exposure (ATSDR, 2007).

For an overview of several cases of human intoxication, see the table below.

***Table:*** *Examples of human intoxication with barium compounds, for which some quantitative information was available.*

| Barium compounds | Dose | Observation | Reference |
|---|---|---|---|
| **Oral** | | | |
| **Barium chloride** | 11 mg/kg bw | Lethal, no further information | RTECS, 1985, referenced by WHO, 2001 |
| **Barium carbonate** | 57 mg/kg bw<br><br>29 mg/kg bw | Lethal, no further information<br><br>Flaccid paralysis, paraesthesia, muscle weakness, no further information | RTECS, 1985, referenced by WHO, 2001 |
| **Barium carbonate** | 13 g, male, 52 years | Recovery after i.v. magnesium sulfate and potassium application and diuresis | Wetherill *et al.,* 1981, also described by MAK, 2010 |
| **Barium carbonate** | Male, 29 years, ~ 1600 mg/kg bw (assuming a body weight of 70 kg), mistaken for barium sulfate | Death occurred within a few hours, preceded by severe signs of intoxication characteristic for barium, no medical intervention | McNally, 1925 |
| **Barium sulfide** | Female, 51 years, ~ 800 mg/kg bw (assuming a body weight of 70 kg), mistaken for barium sulfate | Death occurred within a few hours, preceded by severe signs of intoxication characteristic for barium, no medical intervention | |
| **Barium chloride** | Oral, lethal doses are reported starting at around 3g.<br>Recovery was seen after ingestion of 6.5 g on a full stomach (it is assumed that sulfates present in food render barium insoluble).<br>Higher doses of oral barium up-take were reported and resulted in death within a very short time (minutes to hours). | | |
| **Inhalation** | | | |
| **Barium powder, not specified** | Young male worker | A young chrome-plating worker suffered life-threatening | Shankle and Keane, 1988 |

| | | | |
|---|---|---|---|
| | | hypokalemic paralysis when barium powder used in cleaning the chrome tanks, blew back into his face.<br>RAC note: oral uptake cannot be excluded | |

## 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)**.

### 10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier.

### 10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

### 10.6 Respiratory sensitisation

Hazard class not assessed in this dossier.

### 10.7 Skin sensitisation

Hazard class not assessed in this dossier.

### 10.8 Germ cell mutagenicity

Hazard class not assessed in this dossier.

### 10.9 Carcinogenicity

Hazard class not assessed in this dossier.

### 10.10 Reproductive toxicity

#### 10.10.1 Adverse effects on sexual function and fertility

Since only one study with barium diboron tetraoxide was available for assessment of adverse effects on sexual function and fertility, read-across from repeated dose and reproductive toxicity data on boric acid and borate salts were included in order to support the conclusion on classification.

With the exception of a recent study investigating the effects of boron on rat fertility (Marat et al. 2018) and the study on barium chloride, the studies given in Table 17 below were appointed key studies by the RAC in its 2014 opinions on boric acid, disodium octaborate anhydrate and disodium octaborate tetrahydrate. One human study on the effects of boron on male fertility has been published since March 2014, and is presented in Table 18.

In addition, data on the counter ion from a sub-chronic oral toxicity study on barium chloride in rats were included in Table 17 in order to provide a complete picture of the toxicological profile of barium diboron tetraoxide.

Table 17: Summary table of animal studies on adverse effects on sexual function and fertility

| **Method, guideline, deviations if any, species, strain, sex, no/group**[16] | **Test substance, dose levels duration, of exposure** | **Results** | **Reference** |
|---|---|---|---|
| *Barium diboron tetraoxide* | | | |
| **EPA OPP 82-1 (90-Day Oral Toxicity) and EPA OPP 82-7 (Neurotoxicity)**<br><br>Carried out under GLP and conducted according to US EPA guideline 82-1 and 82-7 but follows OECD TG 408 with the exception of the following organ weights: epididymides, uterus, thymus, spleen.<br><br>Rat (Sprague-Dawley) male/female<br><br>n = 10/sex/group | Test material: Busan 11-M1 (barium metaborate monohydrate)<br><br>Purity: 94.3%<br><br>Form: powder<br><br>Doses/conc.:<br>0, 1000, 5000, 10000 ppm, equivalent to 0, 70, 349 and 707 mg/kg bw in males and 0, 80, 406, and 794 mg/kg bw in females, equivalent to 0, 6.3, 31.4 and 0, 63.6 mg B/kg bw in males and 0, 7.2, 36.5 and 71.4 mg B/kg bw in females, respectively.<br><br>Exposure: 91, 92, 93 or 94 consecutive days prior to necropsy (daily in feed). | **LOAEL for male fertility:** 10 000 ppm (707 mg/kg equivalent to 63.6 mg B/kg bw), based on decreased testes weight and severe aspermatogenesis.<br><br>**1000 ppm (70 mg/kg bw for males and 80 mg/kg bw for females, equivalent to 6.3 mg B/kg bw for males and 7.2 mg B/kg bw for females, respectively):**<br>Males: two males were euthanized *in extremis* (one was hypoactive, unkempt and sacrificed on week 7, and the other one was sacrificed due to a mechanical trauma) and the causes were not attributed to the treatment.<br>No signs of general toxicity were reported for either males or females.<br><br>**5000 ppm (349 mg/kg bw for males and 406 mg/kg bw for females, equivalent to 31.4 mg B/kg bw for males and 36.5 mg B/kg bw for females, respectively):**<br>Males: Statistically significant ($p<0.01$) increased absolute brain weight (by approx. 6%). No statistically significant effects on body weight or body weight gain. No effects on testes weight.<br>Females: statistically significant decrease ($p<0.05$) in body weight during weeks 0-8 and 0-12, compared to controls. The females displayed decreased body weight gain that reached statistical significance ($p<0.05$) only for the 0-8 and 0-12 weeks intervals (by 14% for both time-periods).<br><br>No statistically significant differences as compared to controls were observed in haematology or clinical observations for both males and females.<br><br>**10000 ppm (707 mg/kg bw for males and 794 mg/kg/bw for females, equivalent to 63.6 mg B/kg bw for males and 71.4 mg B/kg bw for females, respectively):**<br>Males: Statistically significant ($p<0.01$) decreased absolute (61%), and relative (57%) weight of testes compared to control. Nine out of 10 males displayed small testes and 7/10 displayed soft testes. Increase (100%) in the incidence of aspermatogenesis (10/10 where 1 was mild and 9 were severe). No spermatocytes present in tubules of the epididymis in 9/10 males. Final body weight decreased with 10% compared to controls ($p<0.05$).<br>Females: Organ weights for either ovaries or uteri were not | Study report 1993a |

[16] Where applicable and unless stated otherwise, the reliability scores of the studies presented in Table 17 are according to the CLH dossier of boric acid, assessed by RAC in 2013.

| **Method, guideline, deviations if any, species, strain, sex, no/group[16]** | **Test substance, dose levels duration, of exposure** | **Results** | **Reference** |
|---|---|---|---|
| | | reported. Histopathological examination of the female organs was not performed. The body weight gain was significantly decreased throughout the whole treatment. (65 – 78 % of that of controls; p<0.05).<br><br>Significantly decreased haematology parameters were observed in both males and females: RBC count (only in males, by 9%; p<0.05), haemoglobin (by 7% in males and 9% in females; p<0.05) and haematocrit (by 7% in males and 10% in females; p<0.05) levels that were considered treatment-related. | |
| *Boric acid* | | | |
| **Sub-chronic oral toxicity (90-day study) (<u>Study 1 and 2</u>)**<br><br><u>Study 1:</u> No guideline specified<br><br>Rat (Sprague-Dawley) male/female<br><br>n = 10/sex/dose group<br><br><u>Study 2</u>: No guideline specified<br><br>Dogs (Beagle) male/female<br><br>n = 5/sex/dose group<br><br>For both studies, survivors were sacrificed after 90 days on the diet. At necropsy the weights of brain, thyroid, liver, spleen, kidney, adrenals and testes were recorded.<br>The tissues preserved in buffered formalin and studied histopathologicall | **For studies 1 and 2:**<br><br>Test material: boric acid or borax<br><br>Purity: unknown<br><br><u>Doses/conc.:</u><br>-Study 1: 0, 52.5, 175, 525, 1750 and 5250 ppm boron, equivalent to 0, 4.7, 15.7, 47.2, 157.5 and 472.5 mg B/kg bw/day, respectively<br><br>-Study 2: 0, 17.5, 175, and 1750 ppm boron, equivalent to 0, 0.4, 4.3 and 43.7 mg B/kg bw/day, respectively<br><br><u>Exposure</u>: 90 consecutive days prior to necropsy (daily in feed).<br><br>**For study 3:**<br><br>Test material: boric acid or borax<br><br>Purity: unknown<br><br><u>Doses/conc.</u>: 0, 117, 350 and 1170 | **<u>Study 1 sub-chronic oral toxicity (rats):</u>**<br><br>**52.5 ppm boron (equivalent to 4.7 mg B/kg bw/day):**<br>One male and one female died during the study.<br><u>Males</u>: no changes in organ weights<br><u>Females</u>: non-statistically significant increased ovary weight (data not shown).<br><br>**175 ppm boron (equivalent to 15.7 mg B/kg bw/day):**<br>No statistically significant changes in growth, body weight, food consumption and organ weights for both males and females.<br><br>**525 ppm boron (equivalent to 47.2 mg B/kg bw/day):**<br><u>Males</u>: partial testes atrophy (5 rats) and spermatogenic arrest (1 rat).<br><u>Females</u>: organ weights comparable to those of control (data not shown).<br><br>**1750 ppm boron (equivalent to 157.5 mg B/kg bw/day):**<br>One male and one female died during the study.<br><u>Males</u>: significantly reduced growth and food utilization efficiency (data not shown, not clear if statistically significant) and a statistically significant (p<0.05) decrease in testes absolute weight (i.e. by approx. 77% for both treatments), accompanied by complete testes atrophy.<br><u>Females</u>: statistically significant (p<0.05) decreased absolute body weight (i.e. 10 – 12 % for both treatments) and absolute ovary weight (p<0.05; by approx. 27% for boric acid treatment, and 42% for borax treatment).<br><br>**5250 ppm boron (equivalent to 472.5 mg B/kg bw/day):**<br>All rats died within 3 to 6 weeks of treatment. For both male and female rats, the necropsy examination showed swollen brain appearance and small gonads for both borax and boric acid treatment (incidence not reported).<br><br>**<u>Study 2 sub-chronic oral toxicity (dogs):</u>**<br><br>**17.5 ppm boron (equivalent to 0.4 mg B/kg bw/day):**<br><u>Males</u>: decreased spleen/body weight ratio (not specified if statistically significant, data not shown) | Weir and Fisher 1972<br><br>Weir 1966 |

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group[16]</th><th>Test substance, dose levels duration, of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>y were brain, pituitary, thyroids, lung, heart, liver, spleen, kidneys, adrenals, pancreas, small and large intestines, urinary bladder, testes, ovary (for rat only), bone and bone marrow.<br><br>Reproduction study (<u>Study 3)</u><br><br>No guideline specified, but conforms to the standard three-generation, 2 litters per generation multi-generation studies normally used at the time.<br><br>The high dose group P1 animals were sterile so only controls, low and mid-dose groups were taken to the F2 and F3 generations.<br><br>Rat (Sprague-Dawley) male/female<br><br>n = 8 males/dose group and 16 females/dose group<br><br>Reliability: 2 (reliable with restrictions)<br><br>Two-year feeding study (<u>Study 4)</u><br><br>No guideline</td>
<td>ppm boron, equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw/day.<br><br><u>Exposure</u>: from the beginning of the study (14 weeks pre-mating exposure) until sacrifice of parents P1, and from weaning until sacrifice of the F1- and F2-generations (daily, in feed).<br><br>For study 4:<br><br>Test material: boric acid<br><br>Purity: unknown<br><br><u>Doses/conc.</u>: 0, 117, 350 and 1170 ppm boron, equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw/day.<br><br><u>Exposure</u>: 24 months, daily in feed.</td>
<td><u>Females</u>: no reported changes in organ weights or organ/body weight ratios.<br><br>175 ppm boron (equivalent to 4.3 mg B/kg bw/day):<br><u>Males</u>: decrease in testes/body weight ratio (not specified if statistically significant, data not shown)<br><u>Females</u>: no decrease in organ weight or organ/body weight ratios.<br><br>1750 ppm boron (equivalent to 43.7 mg B/kg bw/day):<br>One male dog died at day 68 of the study.<br><u>Males</u>: statistically significant decrease ($p<0.05$) in thyroid and testes/body weight ratios (the latter by 40 – 50 % for both treatments), severe testicular atrophy and complete degeneration of the spermatogenic epithelium (4/4 male dogs).<br><u>Females</u>: increased width of the zona glomerulosa of the adrenal glands; markedly atrophied thyroid glands with lymphoid tissue infiltrations for 2 females.<br><br><u>Study 3 reproductive toxicity (rats):</u><br><br>For both low and mid-dose groups, no gross abnormalities for parents or offspring were reported.<br>Significantly ($p<0.05$) higher fertility indices (by approx. 45%, as compared to controls) were reported for F3 generation, for both borax and boric acid treatment.<br><br>The fertility indices for all filial generations (F1, F2 and F3) for both borax and boric acid treatment at 5.9 and 17.5 mg B/kg bw/day are presented below.
<table>
<tr><th>Index</th><th>Control</th><th>5.9 mg B/kg bw/day</th><th>17.5 mg B/kg bw/day</th><th>Control</th><th>5.9 mg B/kg bw/day</th><th>17.5 mg B/kg bw/day</th></tr>
<tr><td colspan="7">Borax</td></tr>
<tr><td rowspan="10">Fertility index [a]</td><td colspan="3">P1-F1A</td><td colspan="3">P1-F1B</td></tr>
<tr><td>62.5</td><td>68.8</td><td>75</td><td>60</td><td>62.5</td><td>75</td></tr>
<tr><td colspan="3">P2-F2A</td><td colspan="3">P2-F2B</td></tr>
<tr><td>81.3</td><td>81.3</td><td>100</td><td>80</td><td>75</td><td>93.8</td></tr>
<tr><td colspan="3">P3-F3A</td><td colspan="3">P3-F3B</td></tr>
<tr><td>68.8</td><td>87.5</td><td>100[b]</td><td>68.8</td><td>87.5</td><td>100[b]</td></tr>
<tr><td colspan="6">Boric acid</td></tr>
<tr><td colspan="3">P1-F1A</td><td colspan="3">P1-F1B</td></tr>
<tr><td>62.5</td><td>87.5</td><td>81.3</td><td>60</td><td>87.5</td><td>75</td></tr>
<tr><td colspan="3">P2-F2A</td><td colspan="3">P2-F2B</td></tr>
<tr><td>81.3</td><td>93.8</td><td>93.8</td><td>80</td><td>93.8</td><td>93.8</td></tr>
<tr><td colspan="3">P3-F3A</td><td colspan="3">P3-F3B</td></tr>
</table>
</td>
<td></td>
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</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group[16]</th><th>Test substance, dose levels duration, of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>specified<br><br>Rat (Sprague-Dawley) male/female<br><br>n = 35/sex/dose group with 70/sex/dose group as controls</td><td></td><td>
<table>
<tr><td></td><td>68.8</td><td>100[b]</td><td>87.5</td><td>68.8</td><td>93.8</td><td>93.8</td></tr>
</table>
[a] Fertility index: number of pregnancies/number of matings x 100.<br>[b] Significantly higher than controls.<br><br>
1170 ppm boron (equivalent to 58.5 mg B/kg bw/day):<br>
All parent groups (P0) were found to be sterile. Only one female (1/16) produced a litter when mated with control males.<br>
<u>P0 males</u>: testes atrophy and lack of viable sperm in all males (8/8 male rats). Reduced body weight with no effect on food intake (data not shown, not clear if statistically significant).<br>
<u>P0 females</u>: decreased ovulation in approx. half of the examined ovaries (data not shown). Reduced body weight with no effect on food intake (data not shown, not clear if statistically significant).<br><br>
<u>Study 4 two-year feeding study (rats):</u><br>
Testes atrophy was observed at 24 months, as shown below:
<table>
<tr><th>Dose level (mg B/kg bw/day)</th><th>0</th><th>5.9</th><th>17.5</th><th>58.5</th></tr>
<tr><td>No. of animals</td><td>3/10</td><td>1/10</td><td>4/10</td><td>10/10</td></tr>
</table>
At 58.5 mg B/kg bw/day, seminiferous tubular degeneration and testicular atrophy were observed at 6, 12 and 24 months of treatment. Based on the observed findings, the LOAEL for fertility in rats was set at 58.5 mg B/kg bw/ day and the NOAEL for fertility in rats was 17.5 mg B/kg bw/day.
</td><td></td></tr>
<tr><td>Study investigating the testicular toxicity of boric acid (BA)<br><br>No guideline specified<br><br>Rat (Fischer 344) male<br><br>n = 6/dose group<br><br>Rats in control and 4500, 6000, and 9000 ppm BA dose groups (n = 96, above) were placed on control NIH-31 pelleted feed after 9 weeks</td><td>Test material: boric acid<br><br>Purity: 99.99%<br><br>0, 3000, 4500, 6000 and 9000 ppm boric acid, equivalent to 0, 525, 788, 1050 and 1575 ppm boron (0, 26, 38, 52 and 68 mg B/kg bw/day), respectively.<br><br>Exposure: 9 weeks (daily in feed)</td><td>
3000 ppm boric acid (equivalent to 26 mg B/kg bw/day):<br>
Mildly inhibited spermiation (Grade 1, i.e. 25 – 50 % tubules at stages below the inhibited spermiation and stage IX with retained spermatids, 0% tubules with germ cell exfoliation and 0% atrophic tubules) by week 5 that continued variably to week 9 (number of males affected not reported). This adverse effect was associated with a testis B level of 5 – 6 µg/g.<br><br>
4500 ppm boric acid (equivalent to 38 mg B/kg bw/day):<br>
Severe and widespread inhibition of spermiation (Grade 2, i.e. >50% tubules at stages below the inhibited spermiation, stage X and XI with retained spermatids, <5% tubules with germ cell exfoliation and 0% atrophic tubules) by week 2 which was maintained up to week 9, when germ cell exfoliation was also observed in <5% of the tubules (number of males affected not reported). This adverse effect was associated with:<br><br>
- a testis B level of 8 – 9 µg/g;<br>
- a variable increase in testicular spermatid head count (TSHC) (24% – 62% at week 2) and no statistically
</td><td>Ku et al. 1993</td></tr>
</table>

| **Method, guideline, deviations if any, species, strain, sex, no/group[16]** | **Test substance, dose levels duration, of exposure** | **Results** | **Reference** |
|---|---|---|---|
| of exposure, and recovery was assessed at 8-week intervals for up to 32 weeks post treatment. Rats were given NIH-31 pelleted feed during the post-treatment period to avoid dental malocclusion problems.<br><br>To assess testis lesion development over time (week 0 – 9) for each dose group, lesions were assigned a numeric score between 0 and 6 (histologic grading scheme), depending on both the lesion characteristics (i.e. atrophic tubules, tubules with germ cell exfoliation, stages with retained spermatids, tubules at stages below the inhibited spermiation) and percentage of tubules affected.<br><br>Reliability: 2 (reliable with restrictions) | | significant changes in testis weight;<br>- a decrease in absolute epididymis weight (10% – 29%) and profound decrease in epididymal sperm count (ESC) (72% – 97%) during weeks 4 – 9.<br><br>The severely inhibited spermiation at 4500 ppm was resolved by 16 weeks post-treatment but areas of focal atrophy that did not recover post treatment were detected.<br><br>**6000 ppm boric acid (equivalent to 52 mg B/kg bw/day):**<br>Initially, severe inhibition of spermiation (not specified if statistically significant, number of males affected not reported) appeared by week 2 which later progressed to severe atrophy (Grade 6, i.e. >95% atrophic tubules). The progression to testicular atrophy was dose-dependent, the rats reached atrophy by week 9. This adverse effect was associated with:<br><br>- a testis B level of 11 – 12 µg/g;<br>- initially increased TSHC (31% – 51%) reflecting the inhibited spermiation at week 2;<br>- progressive and profound decreases in absolute testis weight (12% – 68%) and TSHC (16% – 99%);<br>- decreased absolute epididymis weight (12% - 57%) and decreased ESC (78% - 99%), reflecting the progression to testicular atrophy during weeks 3 – 9.<br><br>No signs of post-treatment recovery from atrophy were observed.<br><br>**9000 ppm boric acid (equivalent to 68 mg B/kg bw/day):**<br>The adverse effects on male fertility at the highest dose level progressed similarly to the 6000 ppm dose level: initially, severe inhibition of spermiation appeared by week 2 (not specified if statistically significant, number of males affected not reported) which later progressed to severe atrophy (Grade 6, i.e. > 95% atrophic tubules). The progression to testicular atrophy was dose- and time-dependent, the rats reached atrophy by week 6. This adverse effect was associated with:<br><br>- a testis B level of 15 – 16 µg/g;<br>- initially increased TSHC (31% – 51%) reflecting the inhibited spermiation at week 2;<br>- progressive and profound decreases in absolute testis weight (12% – 68%) and TSHC (16% – 99%);<br>- decreased absolute epididymis weight (12% - 57%) and decreased ESC (78% - 99%), reflecting the progression to testicular atrophy by week 6.<br><br>No signs of post-treatment recovery from atrophy were observed. | |

| Method, guideline, deviations if any, species, strain, sex, no/group[16] | Test substance, dose levels duration, of exposure | Results | Reference |
|---|---|---|---|
| | | **Feed consumption and body weight gain**<br>Mean (± SD) estimated feed consumptions during weeks 6 and 7: 49.3 - 1.0, 50.2 ± 0.3, 49.2 ± 2.6, 49.2 -+ 1.6, and 44.0 ± 2.1 g/kg body weight/day for 0, 26, 38, 52 and 68 mg B/kg bw/day, respectively. At 68 mg B/kg bw/day, a decrease of 11% in feed consumption and a 16% reduced absolute body weight (controls = 323 ± 6 [SD] g; 9000 ppm = 270 ± 5 g) were observed.<br><br>No changes in body weight gain were observed for the other dose groups, and no other signs of general toxicity were reported. | |
| **Assessing the development of the boric acid-induced testicular lesions by light and electron microscopy**<br><br>No guideline specified<br><br>To determine if there was a hormonal component to the boric acid-induced testicular lesions, serum levels of basal hCG- and LHRH-stimulated testosterone levels were measured. For the tissue boron concentrations, the blood, liver, kidney, epididymis and testis were investigated.<br><br>Rat (Fischer 344), male<br><br>n = 6/time-point (36 male rats in total) for | Test material: boric acid<br><br>Purity: unknown<br><br>Doses/conc.: 0 and 9000 ppm w/w boric acid, equivalent to 0 and 1575 ppm B (0 and 189 mg B/kg bw/day), respectively.<br><br>Exposure: up to 4 weeks (in feed)<br><br>For the histology study and serum testosterone analysis, the animals were euthanised after 4, 7, 10, 14, 21 and 28 days of dosing. | **After 4 days of exposure:**<br>The basal testosterone level was statistically significantly ($p<0.05$) lower than controls (by 65%), and treated and control animals after the hCG- or LHRH challenge. Boron levels had effectively reached steady state levels by day 4 and were not concentrated in the examined tissues.<br>1/6 male rat that presented severely disrupted spermatogenesis and no epididymal sperm, was not included in the analyses.<br><br>**Up to 7 days of exposure:**<br>Inhibition of spermiation and cell sloughing/epithelial disorganisation in approx. 5 – 30% of stage IX tubules appeared in 3/6 male rats. Widespread exfoliation of apparently viable germ cells and pachytene cell death in stages VII and XIV appeared as exposure continued. Statistically significant ($p<0.05$) decreased basal testosterone level (by 85%).<br><br>**Up to 10 days of exposure:**<br>Inhibited spermiation (>60% of tubules) in all stage IX and X tubules was observed in all 6 males. Tubules of stage X, XI and XII (100, 83, and 31%, respectively) contained ≥4 condensed spermatid nuclei near the Sertoli cell basement membranes. Spermatocytes and round spermatids were also seen in the lumina of approximately 10% of all the tubules in 4/6 male rats. Statistically significant ($p<0.05$) decreased basal testosterone level (by 89%).<br><br>**Up to 14 days of exposure:**<br>Inhibited spermiation and peripheral spermatid nuclei (>60% of all tubules) were observed for all rats (6/6). Large, abnormal residual bodies were observed in several stage IX and X tubules. Decreased basal testosterone level (data not reported).<br><br>**Up to 21 days of exposure:**<br>Inhibited spermiation and peripheral spermatid nuclei (>60% of all tubules) were observed for all rats (6/6). Sloughed germ cells occluded the lumina in approx. 30-50% of all tubules in all 6 rats. The number of stage IX – | Treinen and Chapin 1991 |

| Method, guideline, deviations if any, species, strain, sex, no/group[16] | Test substance, dose levels duration, of exposure | Results | Reference |
|---|---|---|---|
| administration of boric acid, and 5/time-point (30 male rats in total) as controls<br><br>Reliability: 2 (reliable with restrictions) | | XII tubules displaying abnormal residual bodies (30 – 60 % of all tubules) was increased for all rats (6/6). Spermatid and spermatocyte cell death was also present in approximately 5 – 30 % of stage VII and XIV tubules. Decreased basal testosterone level (data not reported).<br><br>**At 28 days of exposure:**<br>Inhibited spermiation and peripheral spermatid nuclei (>60% of all tubules) were observed for all rats (6/6). Advanced epithelial disorganization, cell exfoliation (in 70 – 90% of the tubules), luminal occlusion (60 – 80% of the tubules), cell death (30 – 50 % of the tubules) which led to a significant loss of spermatocytes and spermatids from all stage tubules, were observed for 6/6 rats. Statistically significant ($p<0.05$) decreased basal testosterone level (by 69%).<br><br>**Body weights**<br>Over the 28-day study period, the rats consumed approx. 348.3 ± 66.8 mg/kg/day boric acid (mean ± SD). At this concentration, the treated animals gained less weight, at day 28 the treated animals weighed 8% less (statistically significant, $p<0.05$) than the controls (controls = 288 ± 4 g; boric acid = 265 ± 14 g). No other signs of systemic toxicity were reported. | |
| **Reproductive assessment by continuous breeding**<br><br>Performed according to the NTP's Reproductive Assessment by Continuous Breeding Protocol<br><br>Mouse (Swiss) male/female<br><br>n = 19/sex/dose groups<br><br>Sperm concentration was calculated as sperm per mg caudal tissue x $10^3$, the spermatogenic index was used as a semiquantitative | Test material: boric acid<br><br>Purity: >99%<br><br>Doses/conc.: 0, 1000 ppm, 4500 ppm or 9000 ppm equivalent to 0, 152, 636 and 1262 mg boric acid/kg bw, equivalent to 0, 26.6, 111.3 and 221 mg B/kg bw, respectively.<br><br>Exposure: 27 weeks (daily in feed) | **LOAEL (F0) for fertility in mice:** 1000 ppm boric acid (equivalent to 26.6 mg B/kg bw), based on statistically significantly lower sperm motility<br><br>**1000 ppm (equivalent to 26.6 mg B/kg):**<br>F0: The fertility index for 1 – 4 litters was 100%, and 84% for the fifth litter. The F0 males showed statistically significantly lower sperm motility than controls (i.e. 69 ± 5% for treated mice vs. 78 ± 3% for the controls), in 19/19 males.<br>The histopathological exam did not reveal any significant changes for male mice; no histopathological results reported for F0 female mice.<br><br>**4500 ppm (equivalent to 111.3 mg B/kg):**<br>F0: The number of females producing litters decreased from 95% for the production of the first litter, to 85% for the second litter, to 30% for the third litter, to 5% for the fourth and fifth litter. In the female mice, there were no statistically significant changes on body weight, absolute or relative uterus weight; and vaginal cytology revealed normal cyclicity.<br>In the male mice, the following statistically significant ($p<0.05$%) effects were reported, as compared to controls:<br>- decreased mean sperm concentration (by approx. 72%);<br>- decreased mean percentage of motile sperm (by approx. 32%);<br>- increased mean percentage of abnormal sperm (by approx. 439%); | Fail et al. 1991 |

| **Method, guideline, deviations if any, species, strain, sex, no/group[16]** | **Test substance, dose levels duration, of exposure** | **Results** | **Reference** |
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| rating of cell types present, and a quantitative assessment of the number of late spermatids per testis was calculated as number of spermatids per gram of testis x $10^4$.<br><br>Reliability: 2 (reliable with restrictions) | | - decreased seminiferous tubular diameter (by approx. 32%);<br>- decreased number of spermatids in stages VII and VIII/tubule (by approx. 50%);<br>- decreased spermatogenic index (by approx. 28%);<br>- decreased absolute testis weight (by approx. 51%);<br>- decreased absolute epididymis weight (by approx. 21%);<br>- decreased prostate absolute weight (by approx. 20%).<br><br>No statistically significant changes on body weight were observed.<br>The histopathological exam performed in F0 male mice revealed degenerative changes in the majority of the tubules, fewer germ cells that were not organised into the layered epithelium and few mature spermatozoa were observed (incidence not reported).<br><br>**9000 ppm (equivalent to 221 mg B/kg):**<br><u>F0:</u> None of the F0 pairs was fertile.<br>In the male mice, the following statistically significant ($p<0.05\%$) effects were reported, as compared to controls:<br>- decreased mean sperm concentration (by approx. 95%), 12/15 males had no sperm;<br>- decreased seminiferous tubular diameter (by approx. 63%);<br>- no stage VII and VII spermatids/tubule (incidence not reported);<br>- decreased number of spermatids/testis (x $10^4$) by approx. 65%;<br>- decreased absolute testis (by approx. 86%);<br>- decreased absolute epididymis weights (by approx. 34%).<br><br>Histologic examination revealed marked seminiferous tubular atrophy with many tubules per testis characterised by an end-stage, Sertoli cell-only appearance in male rats (100% incidence).<br>No histopathological results reported for F0 female mice.<br><br>The absolute body weight in males was significantly decreased (by approx. 16%; $p<0.05$). The average body weight gain was significantly decreased as compared to controls for both males and females (data not shown). | |
| **Assessment of the fertility of rats exposed to boric acid during spermatogenesis**<br><br>No guideline specified (conforms to Rodent Dominant Lethal Test) | Test material: boric acid<br><br>Purity: unknown<br><br>0, 1 and 10 mg B/kg bw/day<br><br>Exposure: 60 days, daily oral gavage | No information on general toxicity was available for any of the dose groups.<br><br>**1 mg B/kg bw /day**<br>The fertility index was not different from control (86% versus 89% in controls).<br><br>**10 mg B/kg bw/day**<br><br>Reduced fertility index (62.5% compared to 89% in controls, unclear if statistically significantly different).<br>Increased pre-implantation loss (23.81% compared to | Marat et al. 2018 |

| **Method, guideline, deviations if any, species, strain, sex, no/group[16]** | **Test substance, dose levels duration, of exposure** | **Results** | **Reference** |
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| Rats (white outbred),<br><br>n = 6 males/dose group<br><br>Males were administered test substance during the entire spermatogenesis cycle. At the end of the exposure period, the males were mated with untreated females at a 1:1 ratio. Gestation was terminated at day 20 and number of implantation sites, resorptions, and embryos on the uterine horns and the corpus luteum count in the ovaries were investigated.<br><br>The fertility index (FI) was calculated as a ratio of the number of pregnant females to the number of mated females. In a parallel series of experiments, the ability of the test substance to induce mutations in germ and somatic cells was investigated after i.p administration of male rats and frequencies of dominant lethal mutations were also investigated using sequential mating intervals. | | 2.69% in control, $p \leq 0.05$). | |
| *Borax (disodium tetraborate decahydrate)* | | | |

| **Method, guideline, deviations if any, species, strain, sex, no/group[16]** | **Test substance, dose levels duration, of exposure** | **Results** | **Reference** |
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| **Fertility assessment of male rats**<br><br>No guideline specified<br><br>Rat (Sprague Dawley) male<br><br>n = 18 males/dose group<br><br>At the end of the 30 and 60 days exposure periods, 5 male rats from each dose group were serially mated with untreated female rats, in order to assess fertility. Pregnancy rates were calculated as percentage of pregnant females/number of vaginal plugs.<br><br>Reliability: 2 (reliable with restrictions) | Test material: Borax (disodium tetraborate decahydrate)<br><br>Purity: unknown<br><br>Doses/conc.: 0, 500, 1000 and 2000 ppm borax, equivalent to 0, 50, 100 and 200 mg B/kg bw/day, respectively.<br><br>Exposure: 30 and 60 days (daily in diet) | **After 30 days of exposure:**<br>500 ppm borax (equivalent to 50 mg B/kg bw/day): No statistically significant changes in the body, epididymis or testis absolute weight, and no morphological changes observed at the testicular histology examination.<br><br>1000 ppm borax (equivalent to 100 mg B/kg bw/day): Statistically significant ($p<0.05$) decreased absolute epididymis weight (by approx. 19%), marked reduction of spermatocytes, spermatids and mature spermatozoa (incidence not reported).<br><br>2000 ppm borax (equivalent to 200 mg B/kg bw/day): Statistically significant ($p<0.05$) decreased absolute epididymis weight (by approx. 30%), severe loss of germinal elements and non-statistically significant loss in tubular diameter (by approx. 15%).<br><br>Serial mating: no statistically significant changes were observed at 50 mg B/kg bw/day. At 100 mg B/kg bw/day, the pregnancy rates were significantly reduced during the first 3 weeks post-treatment (by 33%; $p<0.05$).<br>At 200 mg B/kg bw/day, the pregnancy rate was statistically significantly ($p<0.05$) reduced (by 100 %) up to 8 weeks after the termination of exposure, with a partial recovery observed up to week 10 post-treatment.<br><br>**After 60 days of exposure:**<br>500 ppm borax (equivalent to 50 mg B/kg bw/day): No statistically significant changes in the body, epididymis or testis absolute weight. A statistically significant ($p<0.05$) decrease (by approx. 16%) in seminiferous tubular diameter was observed, but no morphological changes were observed at the testicular histology examination.<br><br>1000 ppm borax (equivalent to 100 mg B/kg bw/day): Statistically significantly ($p<0.05$) decreased absolute testis weight (by approx. 62%) and absolute epididymis weight (by approx. 37%); most germinal elements were absent (incidence not reported) and a statistically significant decrease (by approx. 34%) in seminiferous tubular diameter was observed.<br><br>2000 ppm borax (equivalent to 200 mg B/kg bw/day): Statistically significantly ($p<0.05$) decreased absolute testis (by approx. 65%) and absolute epididymis weight (by approx. 34%), a statistically significant decrease (by approx. 38%) in seminiferous tubular diameter, and complete germinal aplasia (incidence not reported) were observed.<br>Testicular histology examination 32 weeks post-treatment showed persistent germinal aplasia (incidence not reported). | Lee et al. 1978 |

| Method, guideline, deviations if any, species, strain, sex, no/group[16] | Test substance, dose levels duration, of exposure | Results | Reference |
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| | | A significant decrease in absolute liver weight at 100 and 200 mg B/kg bw/day as compared to controls, with no liver histological changes was observed (by approx. 19% and 25%, respectively; $p<0.05$).<br>A statistically significant ($p<0.05$) dose-dependent increase in the mean plasma FSH concentration by 139%, 175% and 236% for the 500 ppm, 1000 ppm and 2000 ppm dose groups, respectively, was observed after 60 days exposure.<br><br>Serial mating: the pregnancy rates at the mid-dose level were significantly low during weeks 2 – 4 post-treatment (by approx. 80 – 100%), and the males from the highest dose groups were infertile throughout 12 weeks post-treatment (and additional 20 weeks) of serial mating. No statistically significant changes were observed at 50 mg/kg bw/day. | |
| *Barium chloride* | | | |
| **Sub-chronic oral toxicity study and reproductive toxicity screen study**<br><br>No guideline specified<br><br>Rat (Fischer 344) male/female<br><br>Mice (B6C3F1) male/female<br><br>n = 10/sex/dose group/species in the sub-chronic oral toxicity study<br><br>n = 20/sex/dose group/species in the reproductive toxicity screening study<br><br>Differences when comparing to OECD TG 421: dosing only prior to mating, no individual animal data/tables provided, | Test material: barium chloride<br><br>Purity: 99.5%<br><br>**Sub-chronic oral toxicity study:**<br><br>Doses/conc.: 0, 125, 500, 1000, 2000 and 4000 ppm barium chloride dehydrate, equivalent to:<br><br>- for rats: 0, 11.25, 45, 90, 180 and 360 mg/kg bw/day, respectively;<br><br>-for mice: 0, 18.75, 75, 150, 300 and 600 mg/kg bw/day, respectively<br><br>Exposure: 92 days (daily in drinking water).<br><br>**Reproductive toxicity screening study:**<br><br>Doses/conc.: 0, 1000, 2000, and 4000 ppm barium | **Sub-chronic exposure results for rats:**<br><br>No statistically significant changes in absolute body weight were reported for the 11.25, 45, 90 and 180 mg/kg bw/day, for either male or female rats.<br><br>4000 ppm (equivalent to 360 mg/kg bw/day):<br><br>Three of 10 males and 1 of 10 female rats died during the last week of the study. Body weights of both sexes were statistically significantly ($p < 0.05$) lower (by approx. 12% for males, and approx. 9% for females) than controls. Kidney lesions observed in both males and females (incidence not reported).<br><br>**Sub-chronic exposure results for mice:**<br><br>No statistically significant changes in absolute body weight were observed for the 18.75, 75, 150 and 300 mg/kg bw/day dose groups, for either male or female mice.<br><br>4000 ppm (equivalent to 600 mg/kg bw/day)<br><br>Six males and 7 female mice died on day 13 of the study. Body weights of both sexes were statistically significantly ($p < 0.05$) lower (by approx. 30% for males, and approx. 44% for females) than controls. Mild to marked toxic nephrosis was observed in both males and females (incidence not reported).<br><br>**Reproductive and fertility assessment results for rats:**<br><br>NOAEL for fertility impairment: 4000 ppm, equivalent to 480 mg/kg bw/day<br><br>The pregnancy rate§ at 4000 ppm was 65% (compared to 40% in control) and the number of implants per pregnant dam was significantly reduced (7.7 ± 0.52 vs. 9.6 ± 1.10 | Dietz et al. 1992 |

| Method, guideline, deviations if any, species, strain, sex, no/group[16] | Test substance, dose levels duration, of exposure | Results | Reference |
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| histopathologic examination and data on food consumption only provided for core study animals, no humidity and no data on stability of test substance in vehicle were given. Only the average results of the controls and the high dose groups of each species were available.<br><br>Reliability: 2 (reliable with restrictions)[17] | chloride dehydrate for rats, equivalent to 0, 120, 240 and 480 mg/kg bw/day, respectively<br><br>0, 500, 1000, and 2000 ppm barium chloride for mice, equivalent to 0, 90, 180 and 360 mg/kg bw/day, respectively<br><br>Exposure: The males were exposed for 60 days and the females for 30 days (daily in drinking water). | pups in controls, p<0.05). One dam from the highest dose group died, the necropsy revealing 7 foetuses and one resorption site.<br>[§The pregnancy rate was calculated as the number of pregnant females/number of confirmed matings x 100]<br><br>No effects were reported on vaginal cytology, epididymal sperm count, sperm motility, sperm morphology, and testis or epididymal weight up to 480 mg/kg bw/day (data not shown).<br><br>**Reproductive and fertility assessment results for mice:**<br><br>The pregnancy rates ranged from 55 – 70% (the pregnancy rates for the controls were approx. 55%; data not shown) for all dose levels.<br><br>No effects were reported on vaginal cytology, epididymal sperm count, sperm motility, sperm morphology, and testis or epididymal weight up to 360 mg/kg bw/day (data not shown). Maternal weight gain during pregnancy was comparable to controls for all dose groups (data not shown). | |

Table 18: Summary table of human data on adverse effects on sexual function and fertility

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| *Boric acid and borate salts* | | | | |
| **Study type: cohort study (retrospective)**<br><br>Type of population: occupational<br><br>Questionnaire, atmosphere measurement, boron level determination in blood, semen and urine, and determination of semen and sperm parameters.<br><br>Endpoint | The study investigated boron-occupational exposure of workers from a borate-processing plant (Bandirma) and a boron-mining plant (Bigadic Boron Works), both located in Turkey. | HYPOTHESIS TESTED: The global hypothesis was that the means of the five groups are equal (Kruskal-Wallis test).<br><br>METHOD OF DATA COLLECTION<br>Details: A questionnaire survey was carried out to gather information on demographic data and possible confounding variables (age, duration of employment, pesticide application, smoking and alcohol consumption). As lunch was regularly provided for all employees in the central cafeteria, which was located within | Bandirma: Boron concentrations in the drinking water samples taken from the central cafeteria ranged between 16.60 and 45.02 mg B/L.<br><br>Bigadic: The workers who participated in the study were employed at the Bigadic Boron Works and residing in Iskele or Osmanca. Boron concentrations in the drinking water (environmentally) of Iskele were very high, i.e. around 18 mg B/L. Boron concentrations in environmental air samples from the | Duydu et al. 2018a |

[17] The reliability score for this study is according to the publically disseminated REACH Registration dossier for barium chloride, available at https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15037/7/9/2

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| addressed: toxicity to reproduction/fertility | | the boric acid production zone, drinking water and meal samples were taken also from there.<br><br>- Air sampling:<br>**Bandirma: s**tatic air sampling was performed at 5 different stations (central cafeteria/garage, mechanical workshop, steam power plant, infirmary and acid production plant), representing the whole sampling area. Static air sampling was also performed at one air sampling station in downtown Bandirma.<br><br>**Bigadic**: personal air sampling was performed in workers (*n* = 65) working in the high exposure (packaging unit) areas. Static air sampling was performed for the rest of the workers (*n* = 45). Static air sampling was also performed in the village centres of Osmanca and Iskele at two locations, representative of both villages. Both, personal air sampling and static air sampling, were performed using IOM samplers and personal air sampling pumps (SKC, AirCheck 2000). The flow rate was 2 L/min, and the sampling time was 8 h. SKC (GLA-5000), 5 µm, 25 mm filters were used to sample boron compounds within inhalable dust.<br><br>- Biological sampling: performed at the day at which the workers completed their work shift periods (the working programme of the enterprise consisted of | residential areas of Osmanca and Iskele were < LOQ (i.e. 0.9 µg/filter of air samples).<br><br>DBE levels (mg B/day, Mean ± SD (range)):<br>Low exposure group: 15.07 ± 10.50 (3.61–35.61);<br>Medium exposure group: 19.85 ± 15.06 (4.10–47.18);<br>High exposure group: 26.84 ± 15.03 (3.84–55.10);<br>Extreme exposure group: 47.17 ± 17.47 (7.95–106.8).<br><br>Blood boron levels (ng B/g blood, Mean ± SD (range)):<br>Low exposure group: 74.03 ± 28.16 (23.80–99.37);<br>Medium exposure group: 126.6 ± 14.41 (102–149.8);<br>High exposure group: 269.2 ± 73.81 (151–391.9);<br>Extreme exposure group: 570.6 ± 160.1 (402.5–1100).<br><br>Semen boron levels (ng B/g semen, Mean ± SD (range)):<br>Low exposure group: 475.9 ± 639.4 (110.6–2455);<br>Medium exposure group: 1019 ± 1082 (346.7–3863);<br>High exposure group: 1158 ± 1449 (179.4–10543);<br>Extreme exposure group: 1772 ± 1791 (188.7-18072);<br><br>In general, the boron concentrations in the biological fluids were very much paralleled by | |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| | | three work shifts, of 8h each). Peripheral blood samples were drawn from veins of the volunteers into appropriate vacutainer tubes. The blood samples in heparin tubes were stored at 4 °C for subsequent determination of boron. The tubes containing clot activator (BD vacutainer) were used to determine follicle-stimulating hormone (FSH), luteinizing hormone (LH) and total testosterone levels, using Immulite 2000 Immunoassay. The semen samplings and analysis were in accordance with the recommendations of World Health Organization (WHO 2010). Sperm concentration, motility and morphology parameters were determined in fresh semen samples using SQA-V Gold Sperm Quality Analyzer. Spot urine samples (post-shift) were collected in polypropylene containers and stored at –20 °C for subsequent determination of boron and creatinine (Cayman chemical).<br>Analysis of dust collected in cassettes by gravimetric and instrumental methods, boron determination in body fluids was performed with inductively coupled plasma optical emission spectrometry and inductively coupled plasma mass spectrometry.<br><br>STUDY PERIOD:<br>2014 – 2017<br><br>STUDY POPULATION<br><u>- Total population</u>: 212 workers from both Bandirma and Bigadic, classified as follows:<br>Low exposure group: blood boron concentrations < 100 ng B/g blood were ($n$ = 12); | the levels of calculated daily boron exposure (DBE). The correlations between blood boron-DBE, blood boron-urine boron and blood boron-semen boron levels were all statistically significant ($p$ < 0.01). The mean semen boron concentrations of the workers were 6.4, 8.0, 4.3 and 3.1 times higher than the mean blood boron concentrations of workers classified in low, medium, high and extreme exposure groups.<br><br><u>Sperm parameters:</u><br>Sperm quality parameters (and reproductive hormone levels) were compared between the differently exposed groups of workers to identify possible reproductive effects attributable to boron exposure. No statistically significant ($p$ > 0.05) differences were observed in pairwise comparisons of the exposure groups for the following parameters: sperm concentration and sperm morphology parameters (sperm counts, motile sperm, progressively motile sperm concentrations, functional sperm, total sperm number, total motile sperm, total progressive motile sperm, total functional sperm, total number of morphologically normal sperm, percentage of morphologically normal sperm forms). | |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| | | Medium exposure group: with blood boron concentrations between 100 – 150 ng B/g blood (n = 17);<br>High exposure group: with blood<br>boron concentrations between 150 – 400 ng B/g blood<br>(n = 85);<br>Extreme exposure group: with blood boron concentrations ≥ 400 ng B/g blood (*n* = 98).<br><br>- Age and sex of the study population (mean ± SD (range)):<br>Low exposure group (*n* = 12): 33.75 ± 7.85 (24–46), males;<br>Medium exposure group (*n* =17): 35.71 ± 6.75 (27–48), males;<br>High exposure group (*n* = 85): 34.24 ± 6.20 (22–49), males;<br>Extreme exposure group (*n* =98): 36.69 ± 6.52 (23–50), males.<br><br>-Duration of employment (years, mean ± SD (range)):<br>Low exposure group: 4.79 ± 2.37 (2.5–11,0);<br>Medium exposure group: 9.06 ± 7.31 (1–22);<br>High exposure group: 6.33 ± 2.98 (1–15);<br>Extreme exposure group: 6.28 ± 4.76 (1–27).<br><br>- Selection criteria:<br>**Bandirma**: Part of the workers employed at the Bandirma<br>boric acid production zone had been enrolled in the previous<br>"Boron Project I" (Duydu et al. 2011), thus, for the current<br>"Boron Project II" only workers who were not involved in the previous project, were selected. In the current (second) | Sperm motility parameters:<br>The mean values of total motility, progressive motility, non-progressive<br>motility, immotility, velocity, and sperm motility<br>index were compared between the low, medium, high and extreme exposure groups, and no statistically significant difference was observed ($p > 0.05$) in pairwise comparisons of the exposure groups. The mean values of these parameters were again well above their reference values (i.e. according to WHO, the reference values for "total motility" and "progressive motility" are ≥ 40% and ≥ 32%, respectively).<br><br>Hormone levels:<br>FSH, LH and total (free and protein-bound) testosterone concentrations were determined in the blood samples: no statistically significant differences ($p>0.05$) of mean FSH, LH and total testosterone concentrations between the low, medium, high and extreme exposure groups, were found. Statistically significant correlations between blood boron-FSH, blood boron-LH and blood boron-total testosterone concentrations were not apparent<br>($p > 0.05$).<br><br>Conclusions:<br>Boron-mediated adverse effects on semen | |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| | | project,<br>102 workers participated from acid production facilities, steam power plant, mechanical workshop, garage, steelyard, demineralized water production unit, construction units and central cafeteria (cooks), but not from the boric acid production facilities.<br><br>**Bigadic**: In total, 110 workers participated in the study, employed at the Bigadic Boron Works and residing in Iskele or Osmanca (these villages are located near the boron deposits).<br><br>MEASURED PARAMETERS:<br>-DBE (daily boron exposure), boron concentrations in biological fluids (i.e. blood, urine, semen), sperm parameters (i.e. concentration, motile sperm concentration, progressively motile sperm concentration, functional sperm concentration, total sperm number, total motile sperm number, total progressive motile sperm number, total functional sperm, total morphologically normal sperm, morphologically normal forms ), sperm motility parameters (i.e. total motility, progressive motility, non-progressive motility, immotility, velocity, sperm motility index), and FSH, LH and total testosterone levels. | parameters and reproductive hormone levels in males have not been observed under extreme exposure conditions. | |
| **Study type: cohort study (retrospective)**<br><br>Type of population: occupational.<br><br>Questionnaire, | The study investigated boron-environmental and occupational exposure (i.e. boric acid and borax) of workers from a borate-processing plant (Bandirma), | HYPOTHESIS TESTED:<br>The null hypothesis for each biologic fluid was that the means of the respective four groups are equal.<br><br>METHOD OF DATA COLLECTION<br>Details: | The high boron contamination (9.47± 0.18 mg B/L) of water sources for cafeteria and infirmary was not anticipated in the planning phase of the study. This “background” exposure lead to relatively high exposure of the | Duydu et al. 2011<br><br>Başaran et al. 2012 |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| atmosphere measurement, boron level determination in blood, semen and urine, and determination of semen and sperm parameters.<br><br>Endpoints addressed: toxicity to reproduction/fertility. | located in Turkey. | - Personal sampling:<br>exposed group only, personal air sampler (SKC, AirCheck 2000), flow rate 2 L/min, sampling time 8 hours; low-ash PVC filters (SKC, 5 37 mm, preweighed) and SureSeal cassettes (SKC, 37 mm).Analysis of dust collected in cassettes by gravimetric and instrumental methods (Selin B (2010) Boron Determination in Body Fluids by Inductively Coupled Plasma Optical Emission Spectrometry and Inductively Coupled Plasma Mass Spectrometry.<br><br>- Area air sampling: control group only: same devices and parameters were used as for the personal sampling but the devices were not carried by individuals, but used statically, to determine an average value for the control workers.<br><br>Biological sampling: taken at the end of a work shift; no samples taken on the first working day of the week or shift period; workers were informed of the importance to avoid a possible contamination (sampling after showering and changing of clothes).<br><br>STUDY PERIOD:<br>not described in detail, exposure periods (years employed, boron blood level based groups):<br><br>Control 15.30 + 8.63<br>Low exposure 16.85 + 7.06<br>Medium 17.21 + 6.77<br>High 13.96 + 8.04<br><br>STUDY POPULATION<br>- Total population (Total no. of persons in cohort from which the subjects were drawn):<br>exposed: 428 workers, 102 participated: boric acid | control group.<br><br>Total average exposure of occupationally exposure exposed workers: 12.08 ± 6.18 mg boron/day).<br><br>Total average exposure of control workers: 5.83 ±1.71 mg boron/day.<br><br>The average daily boron exposure (DBE, in mg B/d) calculated for the reclassified groups are:<br><br>Control 4.68 ± 1.63<br>Low exposure 7.39 ± 3.97<br>Medium 11.02 ± 4.61<br>High 14.45 ± 6.57<br>• Mean calculated daily boron exposure levels (DBE): significantly higher in exposure groups than in the new control group.<br><br>Exposure to boron:<br>• Restricted to the tap water in the infirmary and the cafeteria of the company (oral) and to the atmosphere in the boron production sites (inhalation).<br>• The mean levels of inhaled boron (mg/8 h) 0.23 ± 0.79, 1.15 ± 3.14, 1.47 ± 2.69, and 2.58 ± 4.96 in control, low, medium and high exposure groups respectively. Medium and high exposure group significantly higher than in the control group<br><br>Boron levels in biological fluids:<br>• Mean urine boron levels: 2.59 ± 1.32, 5.01 ± 2.07, 7.03 ± 2.37, and 9.83 ± 5.13 mg/g creat. In control, low, median and high exposure groups. Significantly higher in exposure groups than in | |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| | | production workers (n=57), borax (disodium tetraborate decahydrate) production workers (n=31), sodium perborate production unit workers (n=5), boric acid plus borax (disodium tetraborate decahydrate) production workers (n=5), laboratory workers (n=2), a storage worker (n=1), a mechanic technician (n=1) controls: 432 workers, acid production plant workers (n=28), steam power plant workers (n=17), demineralized water production (DWP) unit workers (n=2), energy suppliers (n=11), mechanical workshop workers (n=19), garage workers (n=14), steelyard workers (n=2), construction service workers (n=3), laboratory technicians (n=3), and office workers (n=3).<br>- Selection criteria: original groups: exposed: all married workers of the plants described above, wishing to participate, were enrolled. Controls: probably matched for age and years of employment (and possibly additional parameters), not described in detail boron blood level based groups: Exposure groups n (204) Re-classification (ng boron/g blood)<br><br>New control group 49 <LOQ (48.5)<br>Low exposure group 72 >LOQ–100<br>Medium exposure group 44 >100–150<br>High-exposure group 39 >150<br><br>Significant background exposure to boron via the diet prepared in the same cafeteria for both groups made a regrouping necessary which was based on the blood boron levels. All participating | the new control group.<br>• Mean blood boron (ng/g) levels: < 48.5, 72.94 ± 15.43, 121.68 ± 15.62, and 223.89 ± 69.49 in control, low, med and high exposure groups, respectively.<br><br>• Calculated DBE levels: positively correlated with the blood boron concentrations of the workers (Pearson corr. Coeff.: 0.635).<br><br>• Urine boron concentrations: positively correlated with the blood boron concentrations of the workers (Pearson corr. Coeff: 0.633).<br><br>• Semen boron concentrations (ng/g): 807.92 ± 1625.58, 1422.07 ± 1939.03, 1482.19 ± 1410.71 and 1875.68.2255.07 ± 2255.07 in control, low, med and high exposure groups.<br><br>• Semen boron concentrations in exposure groups vs. new control group significantly different; the dose response trend was not significant, variations within groups were great.<br><br>• Correlation between semen boron concentration and blood boron concentration: very low (Pearson corr. Coeff.: 0.222).<br><br>Hormone levels:<br>• no significant differences between groups, except for LH, mid dose vs. high dose.<br><br>• Very weak correlation between blood boron | |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| | | workers were re-classified both according to their calculated daily boron exposure levels and to the blood boron levels. For the re-classification of dose groups blood boron levels published in recent epidemiological studies were taken into account. Workers with a blood boron concentration below the LOQ were combined to form the new control group.<br>- Total number of subjects participating in study: 204<br><br>- Sex/age/race: males original groups:<br>Exposed: 42.62 ± 4.76 (range: 28-50) years, Caucasian;<br>Controls: 41.75 ± 6.29 (range: 23-53) years, Caucasian.<br><br>- Smoker/non-smoker: not reported<br><br>- Total number of subjects at end of study: 204<br><br>- Matching criteria: not reported, probably age and years of employment (and possibly additional parameters)<br><br>COMPARISON POPULATION<br>- Type: Control group<br>- Details: The control group was defined as the group which had blood boron levels below the LOQ (level of quantification).<br><br>HEALTH EFFECTS STUDIED<br>-DBE and blood boron concentrations effects on: Sperm concentration parameters, motility parameters of sperm cells, sperm morphology parameters, DNA integrity with COMET assay, hormone levels (FSH, LH, total | concentration and hormone levels (FSH: Pearson corr. Coeff: 0.143; LH: Pearson corr. Coeff: 0.164; total testosterone level: -0.053).<br><br>• No statistical significant difference in testosterone levels between new control group and exposure groups.<br><br>Semen and sperm parameters (including morphology and DNA integrity testes):<br>• No significant difference in parameter tested between the exposure groups and the new control group.<br><br>• Correspondingly only a weak correlation between the percentages of the normal morphology and blood boron levels.<br><br>• Only weak correlation between inhaled boron (mg/8 h) and blood boron (0.279), inhaled boron–semen boron (0.185), and inhaled boron–urine boron (0.106) levels.<br><br>• Boron effects on semen parameters, reproductive hormone levels, or DNA integrity in sperm cells is absent. No significant dose-dependent relationship between reproductive toxicity biomarkers and blood boron concentration. The relatively extreme boron exposure conditions did not result in blood boron concentrations above considered safe.<br><br>The PSA level was not statistically significantly different when groups are compared. | |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| | | testosterone) and total PSA. | Conclusions:<br>- Due to the background exposure via drinking water no clear relation could be found between inhalation exposure and boron levels in biological fluids.<br>- Blood and urine boron levels increased steadily with rising DBE, while semen boron levels failed to follow a steady trend.<br>- Variation in semen boron levels was high.<br>- Boron is accumulated in semen and the concentration factor is the highest at the lowest exposure.<br>- Adverse effects in hormone levels were absent when exposure groups are compared to the new control group.<br>- For any of the semen parameters, a statistically significant difference was not seen between the new control group and exposure groups. | |
| **Study type: cohort study (retrospective)**<br><br>Type of population: general.<br><br>Interview<br><br>Endpoints addressed: toxicity to reproduction / fertility. | The study investigated boron-environmental exposure of residents from 5 villages located near the borate-processing plant Bigadic, Balikesir county, Turkey. | HYPOTHESIS TESTED: Relationships between elevated boron intake and fertility were sought by comparing reproduction in the residents of two Turkish villages with high levels of boron in their drinking water (one with 8.5 to 29 mg B/L and the other with 2.05 to 2.5 mg B/L), with three nearby villages with more typical lower boron levels (0.03 to 0.45 mg B/L). The two high boron villages were designated as Region I, and the three villages with lower boron in the drinking water were designated Region II. In addition to exposure to elevated boron in drinking water, 28.3 % of the probands in Region I were employed in borate mining or processing, | In high boron areas, the average concentrations ranged from 0.7-29.0 mg B/L.<br>In other lower boron areas 0.05- 0.45 mg B/L.<br>Drinking water in 5 supplies from the very low control area of Camlidere had levels <0.1 mg B/L.<br><br>In the high boron exposure region the infertility rate was 3.17 % in the probands and 3.0 % averaged over 3 generations. In the very low exposure control area infertility was 4.48 %, and in the general Turkish population was 3.84 %.<br><br>No difference in fertility was observed between | Sayli et al. 1998 |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| | | whereas in Region II, 11.7 % were so employed. The data on fertility from these two populations was also compared with that from an area with a very low boron concentration in drinking water and no occupational exposure, and also from data for the whole Turkish population.<br><br>STUDY POPULATION<br>- Total population: The group with the high boron exposures in Regions I and II comprised 927 probands and by the use of a pedigree technique covering three generations, fertility data on 5934 marriages were investigated.<br>- Selection criteria: Relationships between elevated boron intake and fertility were sought by comparing reproduction in the residents of two Turkish villages with high levels of boron in their drinking water (one with 8.5 to 29 mg B/L and the other with 2.05 to 2.5 mg B/L), with three nearby villages with more typical lower boron levels (0.03 to 0.45 mg B/L). The two high boron villages were designated as Region I, and the three villages with lower boron in the drinking water were designated Region II. In addition to exposure to elevated boron in drinking water, 28.3 % of the probands in Region I were employed in borate mining or processing, whereas in Region II, 11.7 % were so employed.<br>-Sex/age/race: Males and females; 40 % of the probands were 30-39 y; 35 % 40-60 y; and 15 % < 30 y<br>-Smoker/non-smoker: Smokers and non-smokers<br><br>COMPARISON | 399 men with occupational exposure to boron, and 222 men with similar occupations but not exposed to boron. It was concluded that within the limits of the study, there was no evidence that boron interfered with human fertility and reproduction. | |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| | | POPULATION<br>- Type: Other comparison group: The data on fertility from the study populations was also compared with that from an area with a very low boron concentration in drinking water and no occupational exposure, and also from data for the whole Turkish population. National population of Turkey 49,856 randomly chosen families. The regional population of Camlidere (relatively boron free soils) was 625 families, covering three generations. | | |
| **Study type: cohort study (retrospective)**<br><br>Type of population: occupational<br><br>Questionnaire | The study investigated boron-occupational exposure (i.e. boric acid and borax) of workers from a borate-processing plant (Bandirma), located in Turkey. | METHOD OF DATA COLLECTION<br>- Details : First phase:<br>The questionnaire covered marital status and childbearing properties of the proband, and included the age at marriage, its duration, the period of first conception, the number of pregnancies, births, foetal losses and congenital malformations, and the number and sex of children both alive and deceased. No physical examination was conducted but medical records if available were recorded.<br><br>Second phase:<br>Computerised individual files of all workers as well as all general management people were checked without interview.<br><br>SETTING: Borates plant, prior to or immediately after an 8 h shift.<br><br>STUDY POPULATION<br>- Total number of subjects participating in study:<br>Phase 1: 191<br>Phase 2: 712 | At the first phase of the investigation, 191 workers were interviewed. Among these there were six infertiles of the primary type with a rate of 3.1 %. Boron-unrelated infertile couples among sibs were found to be 2.6 – 3.6 % and 3.2 % for three-generation marriages – none being higher than those revealed in different sets of controls.<br>In the second stage of work, computerised files of all workers of the facility and all employees of the general management sharing the same location were checked without an interview.<br>Twenty-four subjects (3.4 %) out of 712 workers were childless versus 2.7 % among 108 employees and 2.2 % among 91 workers of a distantly located acid plant of the same complex. The differences were not significant.<br>94.2 % of probands had at least 1 living child at the time of inquiry, including one widow and one separated. 307 children were born to proband families of which 50.1 % | Sayli 2003 |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| | | | were males and 49.9 % females, all alive at the time of the investigation, with a sex ratio of 1.0.<br><br>Nine males and 6 female infants were described as deceased early in life. There were 1.7 alive and 0.1 deceased offspring per family. Of 119 interviewed, 32.5 % had 1 child, 56.6 % had 2 children and 8.8 % had 3 children. The remaining 2.3 % had 4 – 7 children. No discussion of foetal losses or congenital malformations were included. | |
| **Cross-sectional descriptive epidemiology study**<br><br>Endpoints addressed: toxicity to reproduction / fertility<br><br>The study was based on interviews with participants who had occupational exposure to boron and a comparison group selected from an environment without significant exposure to boron. | The study investigated occupational boron-exposure of workers from boron mines and processing plants located in the city of Kuandian, China. | This article described the lifestyle patterns of boron mining and processing workers (N = 936) and a comparison group (N = 251) in Northeast China, and explores relationships between boron exposure and reproductive health. An English version of an interview guide addressing areas of work and lifestyle relevant to boron exposure and metabolism was developed by an occupational health research team, translated to Chinese, and translated back, for clarity.<br><br>Modifications incorporated suggestions from local community advisory board and boron industry workers; the translation-back translation process was reapplied and cultural settings and semantic equivalence was attained.<br><br>The environmental boron exposure for the boron works (mean) and the comparison group (mean) were 2.6 – 3.8 mg/L for boron workers and 0.005 – 0.67 mg/L for the comparison group in surface water; 1.2 – 25.1 mg/L in | 34 % of boron workers reported eating in the contaminated work areas.<br><br>Nearly all boron workers (99 %) showered or bathed after work although approximately 10 % redressed in their contaminated clothes.<br><br>Reproductive health outcomes were explored, including delayed pregnancy, multiple births, spontaneous miscarriages, induced abortions, stillbirths and unusual male:female offspring.<br><br>On average, boron workers fathered nearly 2.0 pregnancies compared with 2.1 pregnancies in the control group (P = 0.6). Of the self-reported pregnancies fathered by boron workers, an average of 1.3 resulted in livebirths, compared to an average of 1.4 for the comparison group (P = 0.3).<br><br>A significant difference existed between groups in | Chang et al. 2006 |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| | | boron workers well water and 0.002 – 0.67 mg/L for the comparison group's well water. | delay in pregnancy, defined as the inability to conceive within 1 year of desiring a child, with boron workers experiencing greater delays. However in logistic regression models adjusting for age, education, race, tobacco, alcohol and soybean consumption the difference was no longer statistically significant (P = 0.11) with an odds ratio of 1.7 for boron workers compared to the control group (95 % confidence interval, 0.09 to 3.5). | |
| **Cohort study (retrospective)**<br><br>Endpoints addressed: toxicity to reproduction / fertility.<br><br>Interview / Questionnaire / Record review | The study investigated occupational boron-exposure of workers from a sodium borates mining and production facility located in the Mojave Desert, California. | METHOD OF DATA COLLECTION<br>The fertility data were obtained primarily by self administered questionnaire, and a section of the group by telephone interview. A 10 % sample of questionnaires was checked against the relevant medical insurance records. The work and exposure data were provided from company records.<br><br>STUDY POPULATION<br>- Selection criteria: All male employees at the U.S. Borax mine and production facility in Southern California with more than 6 months service were invited to participate in the study.<br>- Total number of subjects participating in study: Of the 753 eligible male employees with more than 6 months service, 542 (72 %) participated. The demographic data, length of employment, age and year at hire and medical insurance records of the non-participants and the participants were compared and no significant differences were found.<br>- Sex/age/race: Males; wide | There was a highly significant excess of offspring fathered by the male employees at the mine and production facility (529 observed births compared with 466.6 expected).<br><br>A statistically significant excess in the standardised birth ratio (SBR) of 113, significant at $p < 0.01$. The SBR for the workers with 'low' (< 3 mg/m3) exposures was not different from the SBR of those with 'medium' (3 – 8 mg/m3) and 'high' (> 8 mg/m3) exposures, and both exceeded 100. There was no evidence of a relation between exposure and this excess of offspring, nor were there any temporal differences during the more than 30 year period of observation. The SBR was also evaluated in 5 year periods from 1950-1990 and in every period the SBR was greater than 100.<br><br>9% of workers tried unsuccessfully to | Whorton et al. 1994a<br><br>Whorton et al. 1994b |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
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| | | range with average duration of employment in the facility of 16 years; race not specified<br>- Smoker/non-smoker:<br>Smokers and non-smokers.<br><br>EXPOSURE<br>The range of exposure in one year was 2 to 35.7 mg/m$^3$ (sodium borates). Based on an average of 23.2 mg/m$^3$, the authors calculated the average exposure to borate dusts to be 203 mg/day assuming a 7 hour day and a respiratory volume of 8.75 m$^3$ (based on 10 m$^3$ for 8 hours). They assumed an average or usual boron content of 14% of the dust which, for the high exposure group, is equivalent to a mean of 28.4 mg B/d or 0.4 mg B/kg/d for a 70 kg worker. The average exposure for the highest exposure group was 28.4 mg B/day (approximately 0.4 mg B/kg bw/day) for two or more years. The average duration of exposure was 16 years.<br><br>COMPARISON POPULATION<br>- Type: No specific local control group was studied, but the results expressed as the Standardised Birth Ratio (SBR) were compared with the SBR for the general US population adjusted for maternal age, parity, race and calendar year. | conceive for more than one year, which compares with the national average of 15 % of the adult population.<br><br>An excess in the percentage of female offspring (52.7 % compared with 48.8 % expected) were fathered by these male employees, this increase was not statistically significant, and was not due to a deficit of boys since 249 were observed compared with 238 expected. Thus, there was an excess of 11 boys and 51 girls. There was no evidence of an exposure relationship to sodium borate exposures of the fathers and the excess of female offspring, nor were there any temporal differences. There was an inverse relationship between the increase percentage of female offspring and the sodium borate exposures of their fathers. | |

**Table 19: Summary table of other studies relevant for toxicity on sexual function and fertility**

| Type of study/data | Test substance, | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| No other relevant studies for adverse effects on sexual function and fertility were available | | | | |

### 10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

#### 10.10.2.1 Animal studies

**Data on barium diboron tetraoxide**

90-day oral repeated dose toxicity study (Study report, 1993a)

In a repeated dose toxicity study (90-day oral study, EPA guideline) rats (10/sex/dose) were administered 0, 1000, 5000 and 10 000 ppm barium diboron tetraoxide in feed (equivalent to 0, 6.3, 31.4 and 63.6 mg B/kg bw/day in males and 0, 7.2, 36.5 and 71.4 mg B/kg bw/day in females, respectively). The histopathology examination was performed only for the controls and high-dose males and the following effects on the male reproductive system were reported at 63.6 mg B/kg bw/day: absence of spermatocytes in the tubules of the epididymides (90%), increased incidence (100%) of severe aspermatogenesis, increased incidence of small and soft testes (90% and 70%, respectively, as compared to 0% in controls), and significantly decreased absolute and relative weight of testes (by approx. 61% and 57%, respectively; $p<0.05$). The organ weights of ovaries or uteri for the females in the high dose group were not reported.

In the high dose group (63.6 mg B/kg bw/day in males and 71.4 mg B/kg bw/day in females), the final body weight for both males and females was significantly decreased (by 10% as compared to controls; $p<0.05$). At the mid-dose level (equivalent to 34.4 mg B/kg bw/day for males and 36.5 mg B/kg bw/day for females), a significant decrease in body weight gain for females (by 14%; $p<0.05$) during weeks 0-8 and 0-12 of treatment, and a significant increase in absolute brain weight in males (by approx. 6%; $p<0.05$) were seen. At 63.6 mg B/kg bw/day, significantly decreased haematology parameters were reported for both males and females: haemoglobin (by 7% in males and 9% in females; $p<0.05$), haematocrit (by 7% in males and 10% in females) and RBC count only in males (by 9%; $p<0.05$). These observed effects were considered treatment-related. Two male rats were euthanized *in extremis* at the highest dose level. One male was hypoactive and unkempt, displaying lacrimation, soft stool and clear material on mouth and neck at the time of sacrifice (week 7), and the other was sacrificed due to a mechanical trauma. The observed effects were not ascribed to the treatment according to the author of the study.

To conclude, this study presents clear evidence of alterations on the male reproductive system at 63.6 mg B/kg bw/day, manifested as severe aspermatogenesis, absence of spermatocytes in the tubules of the epididymides and significantly increased incidences of small and soft testes, correlated with significantly decreased absolute and relative testes weights. These effects were observed in the absence of marked general toxicity

**Data on boric acid and borate salts**

The assessment of adverse effects on sexual function and fertility of barium diboron tetraoxide is supported with read-across data from studies of oral exposure to boric acid and borate salts. In aqueous solutions at physiological and acidic pH, low concentrations of barium diboron tetraoxide and simple borates such as boric acid and borate salts will predominantly exist as undissociated boric acid. The toxicokinetic and toxicological properties of barium diboron tetraoxide after oral exposure are therefore expected to be similar to those of boric acid and borate salts.

Continuous breeding reproductive toxicity study (boric acid) (Fail et al. 1991)

In the study performed according to NTP guidelines (Reproductive Assessment by Continuous Breeding Protocol), male and female mice were administered 0, 1000, 4500 and 9000 ppm boric acid (equivalent to 0, 26.6, 111.3 and 221 mg B/kg bw/day, respectively) for 27 weeks.

At 26.6 mg B/kg bw/day, F0 male mice displayed significantly lower sperm motility than controls (by approx. 13%; $p<0.05$) in all 19/19 male mice, and no significant changes where revealed by the histopathological examination. The fertility index for the F0 generation was 100% for the first 4 litters and 84% for the fifth litter. No histopathological results were reported for female mice. The absolute body weights of males were comparable to controls (42.11 ± 1.16 vs. 42.24 ± 0.80 in controls). At 111.3 mg B/kg bw/day, statistically significant ($p<0.05$) changes as compared to controls were seen in F0 male mice: decreased mean sperm concentration and mean percentage of motile sperm (by 72% and 32%, respectively), decreased seminiferous tubular diameter (by approx. 32%), increased mean percentage of abnormal sperm (61.17 ± 5.25 vs. 11.34 ± 0.91 in controls, i.e. by approx. 439%), decreased absolute testis, epididymis and prostate weight (by approx. 51%, 21% and 20%, respectively). The histopathology examination revealed degenerative changes in the majority of the tubules, unorganised layered epithelium germ cells and few mature spermatozoa (incidence not reported). The fertility index for the F0 parental generation from the mid-dose group decreased from 95% for the first litter to 85 %, 30% and 5% for the second, third, fourth and fifth litter, respectively. There were no significant changes in body weight, body weight gain or other signs of general toxicity observed in F0 male mice in this dose group. In F0 female mice, vaginal cytology revealed normal cyclicity and no changes on body weight or uterus weight were seen.

The male mice in the high-dose group (221 mg B/kg bw/day) were infertile and displayed statistically significantly decreased absolute testis (by 86%) and epididymis (by 34%) weights. A significant decrease in sperm concentration (by approx. 95%; $p<0.05$) where 12/15 males had no sperm, and severe seminiferous tubular atrophy (100% incidence) that correlated with significantly decreased seminiferous tubular diameter (by approx. 63%; $p<0.05$) were observed. No histopathology results were reported for F0 female mice.

Based on statistically significantly decreased sperm motility in the F0 parental generation, the LOAEL for fertility was set at 1000 ppm boric acid (equivalent to 26.6 mg B/kg bw/day).

In conclusion, dose-dependent effects on male reproductive organs were observed in F0 mice in absence of general toxicity, mainly expressed as decreased sperm motility starting at 26.6 mg B/kg bw/day, decreased sperm concentration, degenerative changes and atrophy of seminiferous tubules and decreased absolute testis and epididymis weights from 111.3 mg B/kg bw/day. Moreover, none of the F0 pairs was fertile at 221 mg B/kg bw/day in the absence of marked general toxicity.

<u>90-day oral toxicity and three-generation reproduction study (boric acid or borax) (Weir and Fisher 1972; Weir 1966)</u>

The sub-chronic oral toxicity studies of boric acid and borax performed in both rats and dogs (study 1 and 2 below, respectively), showed comparable adverse effects on the male reproductive system for both species. The same authors also performed a three-generation reproductive toxicity study in rats (study 3 below).

In study 1, male and female rats were administered 0, 52.5, 175, 525, 1750 and 5250 ppm boron (equivalent to 0, 4.7, 15.7, 47.2, 157.5 and 472.5 mg B/kg bw/day) as boric acid or borax, in feed, for 90 days. At 47.2 mg B/kg bw/day, the male rats displayed partial testes atrophy and spermatogenic arrest (5/10 and 1/10 rats, respectively), and the organ weights of the females were comparable to those of the controls (data not shown). At 157.5 mg B/kg bw/day, significantly decreased testes absolute weight (by approx. 77%; $p<0.05$) and complete testes atrophy were seen for both boric acid and borax treatments, and the females displayed significantly decreased absolute ovaries weight (by approx. 27% for boric acid and 42% for borax treatment; $p<0.05$). At 472.5 mg B/kg bw/day, both male and female rats died within 3 – 6 weeks of treatment. The necropsy revealed effects on the reproductive system of both sexes (i.e. small gonads, incidence not reported). General toxicity was observed as significantly reduced absolute body weights in females (by approx. 10 – 12 %; $p<0.05$) at 157.5 mg B/kg bw/day and reduced growth and food utilisation efficiency in males (not clear if statistically significant).

In study 2, beagle dogs (males and females) were administered 0, 17.5, 175 and 1750 ppm boron (equivalent to 0, 0.4, 4.3 and 43.7 mg B/kg bw/day) in feed, for 90 days. At 4.3 mg B/kg bw/day, a non-statistically significant decrease in testes weight relative to body weight was seen. The males

administered 43.7 mg B/kg bw/day showed severe testicular atrophy with complete degeneration of the spermatogenic epithelium (in 4/4 males), and a statistically significant decrease in testes relative to body weight (i.e. by 40 – 50%, as compared to controls). One male dog died on day 68 of the treatment with borax. The necropsy examination revealed congested kidneys and severe congestion of the mucosa of small and large intestines.

In study 3 (three-generation reproduction study), male and female rats were administered 0, 117, 350 and 1170 ppm boron (equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw/day, respectively). At 58.5 mg B/kg bw/day, both males and females in the P0 parent groups of both borax and boric acid treatments were found to be sterile due to testes atrophy (8/8 male rats), lack of viable sperm (8/8 male rats) and decreased ovulation (incidence not reported). Only 1/16 female from the high dose group produced one litter when mated with control males. No information on the pups was provided. Reduced body weight for both sexes with no effects on food intake were reported (data not shown). No gross abnormalities or body weight changes were seen for the low and mid-dose groups for the filial generations (data not shown). Significantly higher fertility indices were reported for the F3 generation at 5.9 and 17.5 mg B/kg bw/day, for both borax and boric acid treatments (by approx. 45% as compared to controls for both dose levels; $p<0.05$). Based on the adverse effects in the P0 generation, the LOAEL for fertility in rats was set at 58.5 mg B/kg bw/day.

In study 4 (2-year feeding study-as reported in the publically disseminated REACH Registration dossier for boric acid), male and female rats were administered 0, 117, 350 and 1170 ppm boric acid (equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw/day, respectively). Seminiferous tubular degeneration and testicular atrophy was seen after 6, 12 and 24 months of treatment at 58.5 mg B/kg bw/day. At the end of treatment (24 months), the incidence of testicular atrophy was 10%, 40% and 100% at 5.9, 17.5 and 58.5 mg B/kg bw/day, respectively. Based on these findings, the NOAEL and LOAEL for rat fertility were 17.5 and 58.5 mg B/kg bw/day, respectively.

Nine-week oral repeated dose toxicity study (boric acid) (Ku et al. 1993)

Male rats (6 rats/dose group) were administered 0, 3000, 4500, 6000 and 9000 ppm (equivalent to 26, 38, 52 and 68 mg B/kg bw/day) for 9 weeks.

By week 5 of the treatment with 26 mg B/kg bw/day, rats displayed mildly inhibited spermiation (i.e. in 25 – 50% of tubules, incidence not reported), which continued until week 9. This effect was correlated with a 5 – 6 µg B/g testicular level. At 38 mg B/kg bw/day, severe and widespread spermiation (i.e. in > 50% of tubules, incidence not reported) occurred by week 2 and was maintained until the end of the treatment. This latter effect was associated with a boron testicular level of 8 – 9 µg/g and statistically significant decreases in epididymal sperm count (ESC) (i.e. 72 – 97%) and epididymis absolute weight (i.e. 10 – 29%), during weeks 4 – 9.

The testicular lesions observed at the highest dose levels (52 and 68 mg B/kg bw/day) had a similar progression. The initial marked inhibition of spermiation appeared at week 2 and progressed dose-dependently to severe testes atrophy by weeks 9 and 6, respectively.

At 52 mg B/kg bw/day, the male rats displayed adverse effects on the reproductive organs characterised by initially increased testicular spermatid head count (TSHC) (by 31 – 51% for both dose levels), followed by a statistically significant decrease in TSHC (by 16 – 99%) at the end of the treatment. Statistically significant decreases in absolute testes (by 12 – 68%) and absolute epididymis weights (by 12 – 57%), accompanied by a profoundly decreased ESC (by 78 – 99%), were observed. These adverse effects were associated with boron testicular levels of 11 – 12 µg/g.

At 68 mg B/kg bw/day, an initially increased TSHC (by 31 – 51%), statistically significant decreased absolute testes (by 12 – 68%) and epididymis (by 12 – 57%) absolute weights, and decreased ESC (by 78 – 99%) were seen. These effects were associated with boron testicular levels of 15 – 16 µg/g. While post-treatment recovery from severe atrophy did not occur for the highest exposure levels, at 38 mg B/kg bw/day the severely inhibited spermiation was partially reversible 16 weeks after treatment (areas of focal atrophy that did not recover were detected).

At 68 mg B/kg bw/day, general toxicity was observed as decreased absolute body weights (by 16%, as compared to controls) and reduced feed consumption (by 11%, as compared to controls). No feed consumption or body weight changes were reported at 26, 38 or 52 mg B/kg bw/day.

In conclusion, the observed effects on fertility were considered treatment-related. These findings showed that (i) inhibited spermiation did not appear exclusively at high doses and it was expressed at different testicular levels of B than testicular atrophy, (ii) the progression to testicular atrophy was dose-dependent and (iii) a relationship between dietary and testis levels of boron could be established.

28-day oral repeated dose toxicity study (boric acid) (Treinen and Chapin 1991)

Male rats (6/time-point/dose level) were administered 0 and 9000 ppm boric acid (equivalent to 0 and 189 mg B/kg bw/day, respectively), daily (in feed) for 28 days. The development of lesions was assessed through electron microscopy, histology and serum testosterone measurements.

At day 4 of the treatment, 1/6 males showed disrupted spermatogenesis and no epididymal sperm. The basal testosterone level was significantly lower than controls (by approx. 65%; $p<0.05$) for 6/6 males.

At day 7, inhibited spermiation and cell sloughing/epithelial disorganisation were observed for 3/6 males, with a significantly decreased basal testosterone level as compared to controls (by approx. 89%; $p<0.05$). At day 10 of treatment, effects such as inhibited spermiation and peripheral spermatid nuclei were observed in all male rats (6/6).

For days 14, 21 and 28 of treatment, changes such as advanced epithelial disorganisation, significant loss of spermatocytes and spermatids from all stage tubules and cell exfoliation were seen in 6/6 male rats. The basal testosterone levels were significantly decreased (by 65 – 89%; $p<0.05$) for all evaluated time-points. General toxicity was expressed as significantly reduced absolute body weight (by approx. 8%; $p<0.05$), with no other effects reported at any of the investigated time-points.

In conclusion, already after 4 days of treatment of 189 mg B/kg bw/day serum testosterone levels were significantly decreased, and after 7 days inhibited spermiation and histopathological changes in seminiferous tubules were observed with increasing severity and incidences during the treatment period. There were no indications that the adverse effects on the male reproductive organs were secondary to general toxicity.

60-day oral repeated dose toxicity study (boric acid) (Marat et al. 2018)

In a recent study, male rats (6 rats per dose group) were administered 0, 1 and 10 mg B/kg bw/day for 60 days prior to mating. The male rats were mated with untreated females after the cessation of treatment, and the females were sacrificed on GD 20. Decreased fertility indices for both exposure levels (86% and 62.5% vs. 89% in controls, respectively) were seen. Pre-implantation loss was statistically significantly increased at 10 mg B/kg bw/day (23.81% compared to 2.69% in control). There is no information available on clinical conditions, body weights or body weight gains of the animals, and it is therefore not possible to conclude that the observed findings are not a secondary consequence of general toxicity.

30-day and 60-day oral repeated dose toxicity study (borax) (Lee et al. 1978)

Male rats (18/dose group) were administered 0, 50, 100 and 200 mg B/kg bw/day as borax in diet, for a period of 30 or 60 days. At the end of the exposure periods, 5 male rats from each dose group were serially mated with untreated females.

After 30 days of treatment at 100 mg B/kg bw/day, significantly decreased absolute epididymis weight (by approx. 19%; $p<0.05$) and a marked testicular reduction of spermatocytes, spermatids and mature spermatozoa were seen (incidence not reported, not clear if statistically significant). At 200 mg B/kg bw/day, effects such as significantly decreased absolute epididymis weight (by approx. 30%; $p<0.05$), severe loss of germinal elements and a reduced tubular diameter (by approx. 15%; $p>0.05$) were reported. No statistically significant changes in testis or body absolute weight or other signs of general toxicity were seen at any dose level.

After 60 days of treatment, a significant decrease (by approx. 16%; $p<0.05$) in seminiferous tubular diameter, but no body, testis or epididymis changes were observed at 50 mg B/kg bw/day. At 100 mg

B/kg bw/day, significantly decreased absolute testis and epididymis weights (by approx. 62% and 37%, respectively; $p<0.05$) and a reduction in seminiferous tubular diameter (by approx. 34%; $p<0.05$) were seen. The rats at 200 mg B/kg bw/day displayed significantly decreased testis and epididymis absolute weights (by approx. 65% and 34%, respectively; $p<0.05$), decreased seminiferous tubular diameter (by approx. 38%; $p<0.05$) and complete germinal aplasia that persisted up to 32 weeks post-treatment (incidence not reported). Moreover, a correlation between the dose-dependent germinal depletion and the increased plasma FSH concentrations was observed for the 60-day treatment (i.e. statistically significant increase in mean plasma FSH concertation by 139%, 175% and 236% for the 50, 100 and 200 mg B/kg bw/day, respectively, as compared to controls). No statistically significant body or other organ weight changes or other signs of general toxicity were reported at any dose level.

The serial mating results showed significantly reduced pregnancy rates (100%; $p<0.05$) up to 8 weeks after treatment at 200 mg B/kg bw/day for 30 days, with a partial recovery during weeks 9 and 10 after treatment. At 100 mg B/kg bw/day for 30 days, the pregnancy rates were significantly reduced during the first 3 weeks post-treatment (by 33%; $p<0.05$). The pregnancy rates were comparable to controls at the lowest dose level (50 mg B/kg bw/day), after both treatment periods. The high dose males treated for 60 days were infertile (100%) throughout 12 weeks (and additional 20 weeks) post-treatment. At 100 mg B/kg bw/day, no pregnancies were reported during weeks 2 – 3 after the cessation of treatment of 60 days.

To conclude, the reported adverse effects on fertility were observed in the absence of general toxicity (body weight or clinical observations). The dose-dependent germinal aplasia, complete and partially reversible infertility in male rats (at 200 mg B/kg bw/day for 60- and 30-day treatments, respectively), and the decreased epididymis weights are considered treatment-related.

***Summary of animal studies on barium diboron tetraoxide, boric acid and borate salts***

According to Annex I, paragraph 3.7.1.3 of the CLP Legislation, any effect of substances that has the potential to interfere with sexual function and fertility *includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems*. The above presented animal data on barium diboron tetraoxide, boric acid and borate salts show evidence of adverse effects on sexual function and fertility, mainly expressed as:

<u>1) Alterations to the female and male reproductive system</u>

<u>Females</u>

The test guideline 90-day oral repeated dose toxicity study on barium diboron tetraoxide does not provide evidence on alterations to the female reproductive system since organ weights for ovaries or uteri were not reported and no histopathological examination of the female reproductive organs was performed. Furthermore, in the non-guideline 90-day oral repeated dose toxicity study significantly decreased absolute uterus weight (by 27% for boric acid and 42% for borax treatment; $p<0.05$) was seen in female rats at 157.5 mg B/kg bw/day. In the non-guideline three-generation reproductive toxicity study, decreased ovulation was observed in P0 rats at 58.5 mg B/kg bw/day, but the incidence or information on general toxicity in females were not reported.

The available data do not show clear evidence of alterations to the female reproductive system and thus, are considered as supportive information.

<u>Males</u>

The test guideline 90-day oral repeated dose toxicity study on barium diboron tetraoxide shows evidence of adverse effects on fertility at 63.6 mg B/kg bw/day, mainly expressed as severe aspermatogenesis (10/10), absence of spermatocytes from the tubules of the epididymides (9/10), small (9/10) and soft (7/10) testes. These histopathological changes correlated with significantly decreased absolute and relative testes weight (by 61% and 57%, respectively, as compared to controls; $p<0.05$) and were observed in the absence of marked general toxicity.

The NTP-guideline study of boric acid performed in F0 mice revealed dose-dependent adverse effects on the male reproductive system at 26.6 and 111.3 mg B/kg bw/day, observed in the absence of general toxicity. At 26.6 mg B/kg bw/day, sperm motility was significantly lower than controls (by approx. 13%; $p<0.05$). Significant reductions in the mean percentage of motile sperm and mean concentration of sperm (by approx. 32% and 72%, respectively; $p<0.05$) were seen at 111.3 mg B/kg bw/day. Moreover, a marked increase in the percentage of abnormal sperm (by 439%; $p<0.05$) was noted for the mid-dose level. Similar but more severe effects were observed in F0 mice at 221 mg B/kg bw/day, in the presence of general toxicity (significantly decreased body weight by approx. 16% and reduced body weight gain). The mean sperm concentration was markedly reduced (by 95%; $p<0.05$) as compared to controls, where 12/15 male mice had no sperm, and the number of spermatids/testis was statistically significantly reduced by approx. 65%.

Moreover, in the non-guideline 90-day oral repeated dose toxicity study, partial testes atrophy (5/10) and spermatogenic arrest (10/10) at 47.2 mg B/kg bw/day, in the absence of general toxicity was seen in rats and severe testicular atrophy with complete degeneration of the spermatogenic epithelium (4/4) was observed in dogs, at 43.7 mg B/kg bw/day, in the presence of general toxicity (Weir and Fisher 1972; Weir 1966). In the non-guideline three-generation reproductive toxicity study, testes atrophy (8/8) and lack of viable sperm (8/8) were seen in P0 rats at 58.5 mg B/kg bw/day, in the absence of general toxicity.

Severe and widespread spermiation (incidence not reported) and significantly decreases epididymal sperm counts (72 – 97%; $p<0.05$) were seen at 38 and 52 mg B/kg bw/day in the non-guideline nine-week oral repeated dose toxicity study in rats (Ku et al. 1993). However, no information on general toxicity was reported for either of the dose levels.

In the non-guideline 28-day oral repeated dose toxicity study, inhibited spermiation, epithelial disorganisation, cell exfoliation and significant loss of spermatocytes and spermatids were seen at 189 mg B/kg bw/day, in the absence of marked general toxicity. The basal testosterone level was significantly reduced during the whole treatment (by 65 – 89%; $p<0.05$).

Moreover, dose-dependent germinal aplasia, marked reductions of spermatocytes, spermatids and spermatozoa, and reduced tubular diameter were observed at 100 and 200 mg B/kg bw/day, in the absence of general toxicity in the non-guideline 30-day and 60-day oral repeated dose toxicity studies (Lee et al. 1978).

Statistically significantly reduced testis and epididymis weights were consistently reported by both guideline- and non-guideline oral repeated dose toxicity studies, starting from 38 and 52 mg B/kg bw/day, respectively. In rats, decreased absolute epididymis weight (by 10 – 29%) was observed at 38 mg B/kg bw/day and a profound decrease (12 – 68 %; $p<0.05$) in testis weight was seen at 52 mg B/kg bw/day. In dogs, a significant decrease in testes relative to body weight (by approx. 50%; $p<0.05$) was reported at 43.7 mg B/kg bw/day, in the presence of general toxicity.

The significantly decreased testis and epididymis weights in mice (by approx. 51% and 21%, respectively; $p<0.05$) at 111.3 mg B/kg bw/day correlated with the histopathology results that revealed degenerative changes in the majority of tubules, few mature spermatozoa and few germ cells organised into layered epithelium (Fail et al. 1991). These effects were seen in the absence of general toxicity and are considered as a direct effect of the treatment and thus, relevant for classification purposes.

2) Fertility

In the test guideline continuous breeding reproductive toxicity study performed in mice, fertility indices decreased from 95% for the first litter to 85 %, 30% and 5% for the second, third, fourth and fifth litter, respectively, at 111.3 mg B/kg bw/day. None of the F0 pairs were fertile at 221 mg B/kg bw/day (Fail et al. 1991).

In the non-guideline three-generation reproductive toxicity study performed in rats at 58.5 mg B/kg bw/day, the P0 parent groups were sterile (testes atrophy and lack of viable sperm in 8/8 males) and only one female (1/16) produced one litter when mated with control males. In the F3 generation significantly higher fertility indices, as compared to controls (by approx. 45%; $p<0.05$) at 5.9 and 17.5 mg B/kg bw/day were reported. However, it has to be noted that the fertility indices in controls were

unusually low (ranging from 60% - 81.3 %) for all three filial generations. The serial mating of treated male rats with untreated females (30-day oral repeated dose toxicity study) revealed significantly reduced pregnancy rates (by approx. 33%; $p<0.05$) for the first 3 weeks post-treatment at 100 mg B/kg bw/day (Lee et al. 1978). At 200 mg B/kg bw/day, the pregnancy rates were significantly reduced (100%; $p<0.05$) during 8 weeks post-treatment. However, a 50% recovery during weeks 9 and 10 after treatment was observed. Moreover, at 200 mg B/kg bw/day, the males of the 60-day oral repeated dose reproductive toxicity study were infertile during 12 weeks (and additional 20 weeks) after treatment. At 100 mg B/kg bw/day, significantly reduced (by approx. 80 – 100%; $p<0.05$) pregnancy rates were observed during weeks 2 – 4 post-treatment.

**Data on barium chloride**

Non-guideline 90-day oral repeated dose toxicity and reproductive toxicity screening study (barium chloride) (Dietz et al. 1993)

The 90-day oral toxicity study performed in both mice and rats administered 0, 125, 500, 1000, 2000 and 4000 ppm barium chloride (equivalent to 0, 11.25, 45, 90, 180 and 360 mg/kg bw/day, respectively, in rats, and 0, 18.75, 75, 150, 300 and 600 mg/kg bw/day, respectively, in mice) did not reveal clear evidence of adverse effects on reproductive organs. It should be noted that only the average results of the controls and the high dose groups of each species were presented and no individual animal data or histopathology examination results were available.

Within the same study (but on separate groups of animals), the authors conducted a reproductive and fertility assessment during premating for both rats and mice where the males of each species were treated for 60 days and the female rats and mice were treated for 30 days before mating. The dose ranges differed from the sub-chronic study, mice were administered 0, 500, 1000 and 2000 ppm barium chloride (equivalent to 0, 90, 180 and 360 mg/kg bw/day, respectively) and rats to 0, 1000, 2000 and 4000 ppm barium chloride (equivalent to 0, 120, 240 and 480 mg/kg bw/day, respectively). Pregnancy rates for both mice and rats ranged from 55 – 70% in the treated groups, but since the pregnancy rates of control groups of both species were lower or similar (40% in rats and 55% in mice) compared with the treated groups these findings are not considered toxicologically relevant. No histopathological examination was performed in the animals of the reproductive screening study and no other effects were observed on sexual function or fertility of either species.

***Conclusion on animal studies of barium chloride***

Since there was no evidence of adverse effects on reproductive organs in the 90-day repeated dose toxicity study and no impairment of fertility after barium treatment during premating either in rats or mice, the data on barium were not considered further for classification purposes.

### 10.10.2.2 Human data

No information on human exposure to barium diboron tetraoxide was found in the open literature. Therefore, information was read-across from boric acid and borate salts. Several epidemiological studies carried out on occupationally and/or environmentally boron-exposed populations from Turkey, China and United States of America (USA) are described below.

A recent study performed by Duydu et al. 2018a was designed to assess the effects of occupational boron exposure on the male reproductive system, covering workers with blood boron levels higher than 400 ng B/g blood. A total number of 212 workers from a borate-processing plant (Bandirma) and a boron-mining plant (Bigadic Boron Works), both located in Turkey, participated in the study. The authors collected food, water, biological (i.e. blood, semen and urine), static and personal air samples in order to estimate the daily boron exposure (DBE) levels. Based on the calculated blood boron values, the workers were divided into 4 different groups, as follows: low exposure with a DBE of $15.07 \pm 10.50$ ($74.03 \pm 28.16$ ng B/g blood; n = 12), medium exposure with a DBE of $19.85 \pm 15.06$ mg B/day ($126.6 \pm 14.41$ ng B/g blood; n = 17), high exposure with a DBE of $26.84 \pm 15.03$ mg B/day ($269.2 \pm 73.81$ ng B/g blood; n = 85) and extreme exposure with a DBE of $47.17 \pm 17.47$ mg B/day ($570.6 \pm 160.1$ ng B/g blood; n = 98). The measured sperm quality parameters (i.e. motility, morphology and concentration) as well as the measured hormone levels (i.e. luteinizing hormone

(LH), follicle-stimulating hormone (FSH) and total testosterone) did not show any statistically significant differences in pairwise comparisons of the four exposure groups. However, statistical significance was achieved when comparing the blood boron concentrations to the urine and DBE levels (Pearson's correlation, $p<0.01$). The measured semen levels were 6.4, 8.0, 4.3 and 3.1 higher than the blood boron levels of the low, medium, high and extreme exposure groups, which indicates that the male reproductive organs represent an accumulation site for boron. Based on these results, the authors concluded that extreme occupational exposure to boron (i.e. > 400 ng B/g blood) did not adversely affect male fertility. However, the results of this study might have been influenced by limitations such as the small sample size (i.e. n = 12 for the low exposure group and n = 17 for the medium exposure group), the fact that the different exposure groups were assigned based on blood boron concentrations instead of DBE and that the low exposure group was also environmentally exposed to boron through drinking water. Furthermore, based on an average body weight of 70 kg, the extreme DBE values calculated by this study will be 0.67 ± 0.25 mg B/kg bw/day, and the maximum individual DBE (i.e. 106.8 mg B/day) will be converted into 1.52 mg B/kg bw/day, both values being lower than the LOAEL for fertility in rats (58.5 mg B/kg bw/day) and the NOAEL for rat fertility (i.e. 17.5 mg B/kg bw/day), set by the RAC (RAC Opinion on boric acid, 2014). These results are in line with those reported previously by the same authors (Duydu et al. 2011; Basaran et al. 2012) and show that the assessed DBE levels from Turkish workers are not associated with statistically significant changes on semen parameters, FSH, LH and total testosterone levels.

The study conducted by Duydu et al. 2011 investigated the reproductive toxicity of both occupational and environmental boric acid/borates exposure of populations residing in Turkey. Boron levels from workplace air, food, water sources and biological samples such as blood, urine and semen were determined. Only 102 out of 428 workers involved directly in the manufacturing of boron products participated in the study. The calculated DBE level for the high exposure group 14.45 ± 6.57 mg B/day which, based on an estimated average body weight of 70 kg, can be converted into a value of 0.2 ± 0.09 mg B/kg bw/day. The investigated fertility parameters (i.e. motility, concentration and morphology of sperm) indicated that boron exposure does not affect the male reproductive system. The mean luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone concentrations of the exposed group were not statistically significantly different from the control group levels. However, this study presents several limitations that could have influenced the results. Firstly, only 102 (i.e. 24%) of the occupationally exposed workers participated in the study, out of which only 39 constituted the high exposure group. Thus, having such a small sample size leads to low statistical power. Secondly, the control groups were environmentally exposed to boron through drinking water and further along the study these groups were re-constituted according to blood concentrations of boron instead of occupational exposure. Ultimately, as also stated in the RAC Opinion on boric acid (2014), the high boron exposure level in this study was lower than the NOAEL (0.2 ± 0.09 mg B/kg bw/day vs. 17.5 mg B/kg bw/day) set for male rat fertility, and therefore, could explain the absence of any adverse effects on sexual function and fertility.

Basaran et al. 2012 further investigated the reproductive toxicity parameters in the highly exposed workers in the same occupationally exposed population as in the studies described above. Both exposed and control groups were recruited from the same boron mining area (i.e. Bandirma Boric Acid Production Plant), with the same participation rate as the previous study (i.e. 24%). The calculated mean blood boron level in the highly exposed group was 223.89 ± 69.49 ng B/g blood. At the investigated blood boron levels, the authors did not find statistically significant changes on semen parameters, hormone levels (LH, FSH and testosterone) or DNA integrity in sperm cells. However, this study presents the same study design limitations as the ones described above (Duydu et al. 2018a; Duydu et al. 2011).

Sayli et al. 1998 conducted an observational study on residents of different Turkish villages exposed through drinking water to either elevated (8.5 to 29 mg B/L and 2.05 to 2.5 mg B/L) or lower (0.03 to 0.04 mg B/L) boron levels. The authors compared the reproductive history of the residents living in the two boron-exposed regions through gathering data on the pedigrees of the interviewed families (covering three generations), considering the birth of living children as proof of fertility. The study showed infertility rates of 2.34% for the region exposed to elevated levels of boron was 2.34% where

96% of the families had at least one child, while the infertility rate for the region with the lower boron exposure was 2.62% where 96% of the residents had minimum one child. Based on the collected information, the authors concluded that levels of 8.5 to 29 mg B/L and 2.05 to 2.5 mg B/L found in drinking water did not induce any adverse effects on the fertility of the residents living in the exposed villages. In another study (Sayli 2003), the same main author investigated 191 occupationally exposed workers from two borates and acid plants in Turkey. For data collection under the first phase of the study, a questionnaire was used in order to cover marital status and childbearing properties as well as age at marriage, number of pregnancies and the number of children both dead and alive, births, foetal losses and congenital malformations. The second phase of the study consisted of gathering information covering three-generations on certain parameters (duration of job, involved section, wedding year and number and sex of children) through checking the computerised individual files of 712 workers, without conducting any interviews. While only 3.1 % (6 out of 191) first phase workers were childless, approx. 3.4% (24 out of 712) second phase workers had no children. Based on the observed non-statistically significant differences, it was concluded that occupational boron exposure does not impair human fertility. However, the statistical power of this study is lowered by several study design limitations such as small sample size for the first phase of investigation, not clearly describing the selection criteria for the second phase workers, not conducting any laboratory tests or physical examination of the exposed workers and not deriving any DBE level.

Furthermore, Chang et al. 2006 conducted a cross-sectional, descriptive epidemiological study with occupationally exposed male workers coming from boron mines in China and control groups selected from low environmental boron levels. A total number of 936 exposed workers and a comparison group composed of 251 controls participated in the study. Interviews were conducted in order to collect information on the diet, work history, fertility and health history of the participants, while data on reproductive health parameters were gathered through self-reporting. The mean environmental boron exposure in surface water was between 2.6 – 3.8 mg/L and 0.005 – 0.67 mg/L for the exposed workers and control group, and between 1.2 – 25.1 mg/L and 0.002 – 0.67 mg/L in well water for the exposed workers and control group, respectively. No statistically significant differences in reproductive health outcomes (i.e. delayed pregnancies, multiple births, spontaneous miscarriages, stillbirths and tubal or ectopic pregnancies) for the exposed workers were observed. However, several limitations of this study such as self-reporting of data and the fact that no exposure measurements were performed for the wives of the workers, might have influenced the reported results.

The reproductive effects of borates exposure on male workers and the standardised birth ratio (SBR) for fertility assessment were investigated by Whorton et al. 1994a,b in occupationally exposed workers from a borate mining facility located in the state of California, USA. Out of 753 eligible workers, 542 (i.e. 72%) participated in the study by completing questionnaires for data collection. The participants were divided into five different exposure groups according to the following mean borate exposure values: 0.37 mg/m$^3$, 1.34 mg/m$^3$, 2.23 mg/m$^3$, 3.98 mg/m$^3$ and 8.58 mg/m$^3$. The average exposure for the highest exposure group (n = 109) was calculated at a level of 28.4 mg B/day, which based on an estimated average body weight of 70 kg, can be converted into a value of 0.4 mg B/kg bw/day. The SBR for the workers with low (< 3 mg/m$^3$) exposure levels was not different from the SBR of those with medium (3 – 8 mg/m$^3$) and high (> 8 mg/m$^3$) exposure levels, thus the number of offspring did not indicate any boron-induced adverse effects on male fertility. As also stated in the RAC Opinion on boric acid (2014), the highest average daily exposure (0.4 mg B/kg bw/day) was lower than the LOAEL (26.6 mg B/kg bw/day) for mice fertility according to Fail et al. 1991, and below the NOAEL for fertility in rats (17.5 mg B/kg bw/day) set by RAC on the results reported by Weir et al. 1966.

***Conclusion on human studies of boron***

Overall, the available epidemiological data did not show clear boron-induced adverse effects on sexual function and fertility. As described above, the studies had several methodological limitations and were designed to investigate male fertility only. Other limitations are generally small sample sizes and/or decreased participation rates. It should also be noted that the estimated human exposure levels (DBE) of the “high” and “extreme” exposure groups in these studies were considerably lower than the NOAELs and LOAELs reported for both rats and mice fertility. No studies on effects on fertility and sexual function in humans are available at exposure levels corresponding to the animal LOAELs.

Hence, as was also highlighted by the RAC (Opinions on boric acid (2014), disodium octaborate anhydrate (2014) and disodium octaborate tetraborate (2014)) it is concluded that the available human data on fertility and sexual function do not contradict the animal data. The human data is therefore considered as additional information.

### 10.10.3 Comparison with the CLP criteria

In line with the Repr. 1B classification criteria, the available 90-day oral repeated dose toxicity study on barium diboron tetraoxide (Study report 1993a) shows clear evidence of adverse effects on the male rat reproductive system expressed as severe aspermatogenesis, the absence of spermatocytes in the tubules of the epididymides and statistically significant decreased absolute testes weight (by approx. 61%), at a dose of 707 mg barium diboron tetraoxide/kg bw/day (corresponding to 63.6 mg B/kg bw/day).

The findings for barium diboron tetraoxide (significantly decreased weight of testes, aspermatogenesis, absence of spermatocytes in the tubules of the epididymis) are supported by read-across data from boric acid and borate salts showing similar findings (testicular atrophy, lack of viable sperm, seminiferous tubular degeneration), at a level of 63.6 mg B/kg bw/day and 58.5 mg B/kg bw/day, respectively. The alterations to the male reproductive system, which are dose-dependent and consistent across different species (i.e. mice, rats and dogs), are described in several studies of boric acid and borate salts. Moreover, impaired fertility was reported in a multigeneration reproduction toxicity study of boric acid and borax in the rat. At 58.5 mg B/kg bw/day, P0 parent groups were found to be sterile due to testes atrophy (8/8 male rats), lack of viable sperm (8/8 male rats) and decreased ovulation (incidence not reported). Only 1/16 female from the high dose group produced one litter when mated with control males. In addition, in a reproductive toxicity study in mice none of the F0 pairs were fertile at 221 mg B/kg bw/day in the absence of marked general toxicity, also indicating that boron significantly impairs fertility.

In conclusion, the overall weight of evidence of available information, experimental data on the substance itself and a large body of evidence from read-across data on animal studies showing adverse effects of boron on sexual function and fertility, fulfil the classification criteria requirements for barium diboron tetraoxide as **Repr. 1B, H360F**.

Classification Repr. 1A as is not appropriate as it should be based on human data and no human data on barium diboron tetraoxide were available. Moreover, human data on boric acid and borate salts from read-across do not provide clear evidence of adverse effects on sexual function and fertility. Thus, the overall negative human data do not contradict the animal data, and there is no evidence to indicate that the observed effects in animal studies are not relevant for humans.

Classification in Repr. 2 is not justified since the evidence for adverse effects on sexual function and fertility from existing experimental data on barium diboron tetraoxide and read-across from boric acid and borate salts is considered to be clear and not only *some evidence from humans or experimental animals*.

***Concentration limits***

In line with the CLP guidance (2017), concentration limits for effects on sexual function and fertility are derived by calculating the reproductive toxicity dose descriptor, i.e. ED10 (the dose level at which a change of 10% compared to the concurrent control group is observed). It should be noted that, with the exception of testes weight (Study report, 1993a), the available data on barium diboron tetraoxide were not transparently reported enough in order to derive the ED10, and thus read-across data on boric acid and borate salts were used.

According to the RAC (RAC opinions on boric acid, disodium octaborate anhydrate and disodium octaborate tetrahydrate, 2014), testes atrophy was identified as the most sensitive effect on fertility in rats, based upon a 2-year feeding study with boric acid (Weir 1966). At the end of the treatment (24 months), the incidence of testicular atrophy was 30%, 10%, 40% and 100% at 0, 5.9, 17.5 and 58.5 mg B/kg bw/day, respectively. Based upon these results, the ED10 would therefore be 17.5 mg B/kg bw/day (equivalent to 100 mg boric acid/kg bw/day).

Correcting for the percentage of boron, a level of 17.5 mg B/kg bw/day would correspond to 194.4 mg barium diboron tetraoxide/kg bw/day. The medium potency group with a GCL of 0.3% would therefore be assigned to barium diboron tetraoxide since the ED10 is ≥ 4 mg/kg bw/day and ≤ 400 mg/kg bw/day.

***Conclusion***

Setting of specific concentration limit for adverse effects on sexual function and fertility is not considered justified, and thus the GCL of 0.3% applies.

### 10.10.4 Adverse effects on development

Since only one study with barium diboron tetraoxide was found for the assessment of adverse effects on the development of the offspring, read-across from developmental toxicity data on boric acid and borate salts were included to support the conclusion on classification.

One new animal study investigating the reproductive toxicity of boron in rats has been published in 2018 (Marat et al. 2018). With the exception of this new study, the studies on boric acid and borate salts presented in Table 20 were appointed key studies by the RAC in their 2014 opinion documents on boric acid, disodium octaborate anhydrate and disodium octaborate tetrahydrate. Two epidemiological studies assessing the effects of boron on the development of the offspring, which have been published since 2014 (Duydu et al. 2018b and Igra et al. 2016) are presented in Table 21.

In addition, data on the counter ion from a sub-chronic oral toxicity study and a prenatal developmental toxicity study on barium chloride in rats have been included in Table 20 in order to provide a complete picture of the toxicological profile of barium diboron tetraoxide.

Table 20: Summary table of animal studies on adverse effects on development

| **Method, guideline, deviations if any, species, strain, sex, no/group**[18] | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| *Barium diboron tetraoxide* | | | |
| **Prenatal Developmental Toxicity Study**<br><br>GLP-compliant US EPA guideline 83-3, the bodyweights were not measured with the frequency recommended by the guideline.<br><br>Rabbit (New Zealand), female<br><br>n = 20/dose group | Test material: Busan 11-M1 (barium metaborate monohydrate)<br><br>Purity: 94.3%<br><br>Form: powder<br><br><u>Doses/conc.:</u> 0, 2, 10, 20 mg/kg bw/day, equivalent to 0, 0.18, 0.9 and 1.8 mg B/kg bw/day, respectively | **NOAEL (maternal toxicity)**: 10 mg/kg/day, equivalent to 0.9 mg B/kg bw/day<br>**NOAEL (developmental toxicity)**: 20 mg/kg/day, equivalent to 1.80 mg B/kg bw/day<br><br>**Maternal effects**:<br>- at 1.8 mg B/kg bw/day, 1 dam died on GD 16, and at necropsy 3 normally developing implantations and 5 early resorptions were observed *in utero* (no clinical signs were noted in this female during the study);<br><br>- at 1.8 mg B/kg bw/day, 1 dam aborted on GD 22 (this dam was hypoactive on GD 20-21) 7 late resorptions and 2 early resorptions;<br><br>- 0/20, 1/20, 1/20 and 2/18 dams were non-gravid at 0, 0.18, 0.9 and 1.8 mg B/kg bw, respectively; | Study report 1993b |

[18] Where applicable and unless stated otherwise, the reliability scores of the studies presented in Table 20 are according to the CLH dossier of boric acid, assessed by RAC in 2013.

| **Method, guideline, deviations if any, species, strain, sex, no/group[18]** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| Reliability[19]: 1 (reliable without restriction), key study | Exposure: gestation days 7-19 via oral gavage. The animals were observed until gestation day 29 at which point they were sacrificed. | - no statistically significant changes in mean body weight and body weight, mean gravid uterine weights and food consumption were reported at any dose level.<br><br>**Foetuses:**<br>- No statistically significant differences on the mean foetal body weights were reported. The number of foetuses (litters) available for morphological examination were: 124(20), 129(19), 119(19) and 103(16) at 0, 0.18, 0.9 and 1.8 mg B/kg bw, respectively;<br><br>- external malformations were observed in 3, 0, 0, and 1 foetuses at 0, 0.18, 0.9 and 1.8 mg B/kg bw, respectively. In the control group, 2 foetuses with short tail and 1 foetus with omphalocele were reported. One foetus with carpal flexure on the right front limb was reported at 1.8 mg B/kg bw;<br><br>- soft tissue malformations were observed in 1, 0, 0 and 3 foetuses at 0, 0.18, 0.9 and 1.8 mg B/kg bw, respectively. At 1.8 mg B/kg bw, one foetus with diaphragmatic hernia was observed. At 1.8 mg B/kg bw, hydrocephaly (consisting of increased cavitation of the lateral ventricles) was seen in 2 foetuses from separate litters, and, diaphragmatic hernia was observed in 1 foetus at the same dose level. In the control group, 1 foetus with bulbous aorta (ascending and arch along with a stenotic pulmonary trunk) was observed.<br><br>-skeletal malformations were observed in 7, 1, 5 and 3 foetuses at 0, 0.18, 0.9 and 1.8 mg B/kg bw, respectively. These malformations consisted of:<br>- in the control group, vertebral anomalies with/without associated rib anomaly in 6 foetuses, and severely malaligned sternebrae with a rib anomaly in 1 foetus;<br>- an extra site of ossification anterior to sternebra no. 1 was observed in 1 foetus at 0.18 mg B/kg bw;<br>- at 0.9 mg B/kg bw, vertebral anomalies with/without associated rib anomaly in 4 foetuses, and costal cartilage anomaly with fused sternebrae in 1 foetus;<br>- at 1.8 mg B/kg bw/day, vertebral anomalies with/without associated rib anomaly in 2 foetuses, and an extra site of ossification anterior to sternebra no. 1 in 1 foetus. | |
| *Boric acid and borax* | | | |
| **Prenatal Developmental Toxicity Study** | Test material: boric acid<br><br>Purity: 98% | **LOAEL (developmental toxicity):** 13.3 mg B/kg bw/day, based on reduced foetal body weight and increased incidence of short rib XIII | Price et al. 1996a<br><br>Price et al. |

[19] The reliability score for this study is according to the publically disseminated REACH Registration dossier of barium diboron tetraoxide, available at https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15812/7/9/3

| **Method, guideline, deviations if any, species, strain, sex, no/group[18]** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| GLP-compliant<br><br>Rat (Crl: CD VAF/Plus (Sprague Dawley))<br><br>n = groups of 14 - 17 females/dose group/phase<br><br>Reliability: 1 (reliable without restriction), key study<br><br>In phase I the dams were sacrificed on Day 20 for detailed foetal examination.<br><br>In phase II the dams were allowed to deliver and the pups reared to weaning and then killed for full visceral and skeletal examination as for phase I.<br><br>Maternal blood samples were collected at termination on GD 20. Boron concentration in these blood samples was subsequently determined by inductively coupled plasma (ICP) optical emission spectrometry. | <u>Doses/conc.:</u> 0, 250, 500, 750, 1000, 2000 ppm boric acid equivalent to 0, 19, 36, 55, 76 and 143 mg boric acid/kg bw/day, respectively (equivalent to 0, 3.3, 6.3, 9.6, 13.3 and 25 mg B/kg bw/day)<br><br><u>Exposure phase I</u>: days 0 - 20 post mating (nominal in diet)<br><br><u>Exposure phase II</u>: days 0 - 20 post mating (nominal in diet), then on normal diet until termination on day 21 postpartum | **NOAEL (developmental toxicity):** 9.6 mg B/kg bw/day<br><br>**Maternal effects**<br>No maternal deaths occurred and no treatment-related clinical signs of toxicity were observed in the dams, at any dose level.<br>Increasing dietary concentrations of boric acid were positively associated with whole blood boron concentrations in confirmed pregnant rats: 0.229 ± 0.143, 0.564 ± 0.211, 0.975 +/- 0.261, 1.27 ± 0.298, 1.53 ±- 0.546, or 2.82 ± 0.987 µg B/g whole blood for the control through high-dose groups.<br><br>**Effects on the offspring**<br>**Phase I**: Statistically significant reductions in the mean foetal body weight per litter at the two highest dose levels (i.e. by approx. 6 % at 13.3 mg B/kg bw/day and by approx. 13% at 25 mg B/kg bw/day compared to controls).<br>The following skeletal changes were observed:<br>- Statistically significant increase in the incidence of short rib XIII amongst offspring (i.e. by approx. 1.5% at 13.3 mg B/kg bw/day and by approx. 3.4% at 25 mg B/kg bw/day, compared to controls);<br>- Statistically significant increase in the incidence of wavy rib amongst offspring (i.e. by approx. 2.1% at 13.3 mg B/kg bw/day and by approx. 10% at 25 mg B/kg bw/day, compared to controls);<br>At the highest dose (25 mg B/kg bw/day), these changes were more pronounced.<br><br>**Phase II**: No reduction in pup bodyweight in any group at any time point compared to controls. The rib variations observed in the foetuses from Phase I were not observed at any dose group in Phase II.<br>Only at the highest dose in Phase II (25 mg B/kg bw/day), a statistically significant increased incidence of short rib XIII was observed (by approx. 4% compared to controls). | 1997 |
| **Equivalent or similar to OECD TG 414 (Prenatal** | Test material: boric acid<br><br>Purity: unknown | **LOAEL (maternal toxicity):** 44 mg B/kg bw/day, based on reduced food intake, reduced body weight gain and abortions | Price et al. 1996b<br><br>Heindel et |

| Method, guideline, deviations if any, species, strain, sex, no/group[18] | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| **Developmental Toxicity Study)**<br><br>GLP-compliant<br><br>Rabbit (New Zealand White), female<br><br>n = 30 pregnant female rabbits/ treatment group<br><br>Reliability: 1 (reliable without restriction), key study<br><br>The females were sacrificed on GD 30 and the numbers of uterine implantations, resorptions, dead foetuses and live foetuses were examined. | <u>Doses/conc.:</u> 0, 62.5, 125 or 250 mg/kg bw/day boric acid, equivalent to 0, 11, 22 and 44 mg B/kg bw/day, respectively<br><br><u>Exposure</u>: treatment on days 6 - 19 post-mating, via oral gavage | **NOAEL (maternal toxicity):** 22 mg B/kg bw/day<br><br>**LOAEL (developmental toxicity):** 44 mg B/kg bw/day, based on increased resorptions and cardiovascular malformations in surviving foetuses<br><br>**NOAEL (developmental toxicity):** 22 mg B/kg bw/day<br><br>**Maternal effects**<br>One dam from the 101 mg B/kg bw/day group died on GD 25 and one dam from the mid-dose group died on GD 22, but the deaths were not considered treatment-related.<br>A high vaginal bleeding incidence was observed in the highest dose group, where 2 - 11 pregnant females/day bled between GD 19 - 30.<br>At 44 mg B/kg bw/day, the food intake and body weight gain were statistically significantly decreased, by approx. 31% and by approx. 10%, respectively compared to controls.<br><br>**Foetal effects**<br>At 44 mg B/kg bw/day, a statistically significantly increased rate of resorptions per litter (89.9 ± 5.0 %; 73% of all the does had 100% resorptions) was observed. Only 6 litters survived to GD 30 (compared to 18 – 23 litters for the control and other dose levels).<br><br>The incidence of skeletal malformations (i.e. cleft sternum, detached extra rib – lumbar 1, fused sternebrae and fused rib) was increased, but not statistically significantly different from controls (19, 22, 29 and 29% for the controls, 11, 22 and 44 mg B/kg bw/day dose groups, respectively.<br><br>The incidence of visceral malformations (cardiovascular) was 8.2, 6.3, 7.8 and 78.6% in control, 11, 22 and 44 mg B/kg bw/day dose groups. Statistically significant differences compared to control were only seen at the highest dose level, as follows:<br>- interventricular septal defect in 0.6, 1.7, 1.3 and 57% foetuses (control to high-dose group);<br>- enlarged aorta in 0, 0.6, 0.7 and 36% foetuses (control to high-dose group);<br>- papillary muscle malformations in 3, 2, 4 and 14% foetuses (control to high-dose group);<br>- double outlet right ventricle (pulmonary artery and aorta both arising from the right ventricle) in 0, 0, 0 and 14% foetuses (control to high-dose group). | al. 1994 |
| **Prenatal developmental toxicity of boric acid in mice and rats**<br><br>GLP-compliant | Test material: boric acid<br><br>Purity: 98 – 99%<br><br><u>Rats:</u><br><u>Doses/conc.:</u> 0, 0.1, 0.2 or 0.4 % | **NOAEL (developmental toxicity for rats):** < 14 mg B/kg bw/day<br><br>**LOAEL (developmental toxicity for rats):** 14 mg B/kg bw/day, based on statistically significantly reduced average foetal body weight<br><br>**NOAEL (developmental toxicity for mice):** 43 mg B/kg | Heindel et al. 1992 |

| **Method, guideline, deviations if any, species, strain, sex, no/group[18]** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| Cesarean-originated, barrier-sustained CWDl (ICR) VAF/Plus outbred Swiss albino (CD-l) mice<br><br>Crl:CD BR VAF/:Plus outbred Sprague-Dawley (CD) rats<br><br>n = 26 – 28 female mice or rats/dose group<br><br>Reliability: 2 (reliable with restrictions) key study | and 0.8% equivalent to 0, 78, 163, 330 and 539 mg boric acid (mg B)/kg bw/day, equivalent to 0, 14, 29, 58 and 94 mg B/kg bw/day, respectively<br><br><u>Mice:</u><br><u>Doses/conc.:</u> 0, 0.1, 0.2 or 0.4 % equivalent to 0, 248, 452 and 1003 mg boric acid/ kg bw/day, equivalent to 0, 43, 79 and 175 mg B/kg bw/day, respectively<br><br>Exposure (daily in feed):<br><br><u>Rats:</u> GD 0 – 20 for the dose levels of 14 up to 58 mg B/kg bw/day;<br>GD 6 – 15 only for the highest-dose level (i.e. 94 mg B/kg bw/day), with a separate control group with the same exposure time;<br><br><u>Mice:</u> GD 0 – 17 | bw/day<br><br>**LOAEL (developmental toxicity for mice):** 79 mg B/kg bw/day, based on statistically significantly reduced foetal body weight and increased incidence of skeletal malformations (i.e. short rib XIII)<br><br>**Observed effects in rats**<br>*Maternal effects*:<br>Statistically significant increases compared to control:<br>-relative liver weight by 5% and by 6%, at 29 and 58 mg B/kg bw/day, respectively;<br>-relative kidney weight by 11% and by 12% for 29 and 58 mg B/kg bw/day, respectively.<br><br>Statistically significantly decreased body weight by 11% and by 35%, at the dose levels of 58 and 94 mg B/kg bw/day, respectively, compared to controls.<br><br>*Embryo/foetal effects*:<br>Statistically significantly increased prenatal mortality at 94 mg B/kg bw/day (36% resorptions/litter compared to 4% for the controls).<br><br>Statistically significantly reduced average foetal body weight for all treated groups compared to controls:<br>- 7% decrease at 14 mg B/kg bw/day;<br>- 13 % decrease at 29 mg B/kg bw/day;<br>- 37 % decrease at 58 mg B/kg bw/day;<br>- 50 % decrease at 94 mg B/kg bw/day.<br><br>Increased incidences of malformations were observed:<br>- malformations of the eyes (i.e. displaced eye in 7/136 foetuses and convoluted retina in 9/136 foetuses) at 94 mg B/kg bw/day, compared to the and 0/215 in the control group;<br>- enlarged lateral ventricles of the brain (in 21/386 foetuses at 58 mg B/kg bw/day and in 36/136 foetuses at 94 mg B/kg bw/day), compared to the respective control groups, 0/431 and 0/215;<br>- agenesis of rib XIII was observed in 24/386 foetuses at 58 mg B/kg bw/day and in 17/136 foetuses at 94 mg B/kg bw/day, compared to the respective control groups, 1/431 and 0/215.<br><br>Statistically significantly increased incidence of short rib XIII observed in 39% and 37% of the foetuses at 58 mg B/kg bw/day and 94 mg B/kg bw/day, respectively (compared to their respective control groups, 0.23% and 0.46%).<br><br>Statistically significantly increased incidence (100%) of litters with 1 or more foetuses with a skeletal malformation was reported for both 58 mg B/kg bw/day and 94 mg B/kg bw/day dose levels (24/24 litters and 14/14 litters, respectively compared to their respective control groups, | |

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group[18]</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>4/28 and 2/14).<br><br>Statistically significantly increased incidence of foetuses with visceral or external malformations for all dose groups compared to controls.<br>At 29 and 58 mg B/kg bw/day, increases in incidence of foetuses with visceral or external malformations of 8% and 50% compared to 2% for the control group, respectively, were reported. The incidence of foetuses with visceral or external malformations was statistically significantly increased for the highest dose level (i.e. 73% at 94 mg B/kg bw/day as compared to 2.79% in the control group).<br><br>Observed effects in mice<br>Maternal effects:<br>At 175 mg B/kg bw/day, maternal body weight was statistically significantly reduced (by approx. 25%) during the treatment period.<br>A dose-related increase in the incidence of renal tubular dilation was observed at microscopic examination.<br>At the dose levels of 43 and 175 mg B/kg bw/day, ovarian cysts were seen in 1 dam of each dose group.<br><br>Embryo/foetal effects:<br>At 175 mg B/kg bw/day, statistically significantly increased resorptions (approx. 19% per litter compared to 6% in controls) was observed.<br>Statistically significantly reduced foetal body weights were observed at 79 and 175 mg B/kg w/day (by approx. 12% and 33%, respectively compared to controls).<br>At the 175 mg B/kg bw/day, a statistically significantly increased incidence (approx. 8%) in foetuses with malformations as compared to the control groups (approx. 2%) was reported.<br>Statistically significantly increased incidence of short rib XIII was observed in 10/250 foetuses at 175 mg B/kg bw/day, compared to 0/311 in controls. Agenesis of one or more vertebra (lumbar) was reported for 3/250 foetuses (as compared to 1/311 in controls) for the highest dose level.</td><td></td></tr>
<tr><td>Reproductive toxicity assessment study<br><br>No guideline specified, but conforms to the standard three-generation, 2 litters per generation multi-generation studies normally used at the time.<br><br>The first filial</td><td>Test material: boric acid or borax<br><br>Purity: unknown<br><br>Doses/conc.: 0, 117, 350 and 1170 ppm boron, equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw<br><br>Exposure: from</td><td>For all filial generations (i.e. F1, F2 and F3), for both low- and mid-dose groups, the litter size, weights of progeny and appearance were not statistically significantly different from controls (data not shown). No information on maternal toxicity is reported.<br>At 58.5 mg/kg bw/day there were no offspring produced from P1 animals.<br><br>The live birth indices for both boric acid and borax treatment, at 5.9 and 17.5 mg B/kg bw/day are presented below:<br><br>
<table>
<tr><th>Index</th><th>Control</th><th>5.9 mg B/kg bw/day</th><th>17.5 mg B/kg bw/day</th><th>Control</th><th>5.9 mg B/kg bw/day</th><th>17.5 mg B/kg bw/day</th></tr>
</table>
</td><td>Weir and Fisher 1972</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group[18]</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>generation (F1A) was carried through weaning and discarded. The parental generation (P1) was rebred to produce their second litter (F1B). At the time of weaning, 16 females and 8 males each from the control and test groups were selected at random and designated the second parental generation (P2) for continuation of the reproduction study. These animals were bred to produce the F2A and F2B litters as before. The F2B litter became the P3 generation and were bred to produce the F3A and F3B litters.<br><br>Rat (Sprague-Dawley) male/female<br><br>n = 8 males/dose group and 16 females/dose group<br><br>Reliability: 2 (reliable with restrictions)</td><td>the beginning of the study (14 weeks pre-mating exposure) until sacrifice of parents P1, and from weaning until sacrifice of the F1- and F2-generations (daily in feed).</td><td>
<table>
<tr><th colspan="7">Borax</th></tr>
<tr><td rowspan="12">Live birth index[a]</td><td colspan="3">P1-F1A</td><td colspan="3">P1-F1B</td></tr>
<tr><td>98.4</td><td>98.4</td><td>100</td><td>99.1</td><td>99.2</td><td>99.4</td></tr>
<tr><td colspan="3">P2-F2A</td><td colspan="3">P2-F2B</td></tr>
<tr><td>97.8</td><td>99.4</td><td>96.9</td><td>98.6</td><td>92.4</td><td>98.8</td></tr>
<tr><td colspan="3">P3-F3A</td><td colspan="3">P3-F3B</td></tr>
<tr><td>100</td><td>100</td><td>99.4</td><td>100</td><td>100</td><td>100</td></tr>
<tr><td colspan="7">Boric acid</td></tr>
<tr><td colspan="3">P1-F1A</td><td colspan="3">P1-F1B</td></tr>
<tr><td>98.4</td><td>96</td><td>97.2</td><td>99.1</td><td>99.4</td><td>100</td></tr>
<tr><td colspan="3">P2-F2A</td><td colspan="3">P2-F2B</td></tr>
<tr><td>97.8</td><td>100</td><td>99.4</td><td>98.6</td><td>99.4</td><td>97.9</td></tr>
<tr><td colspan="3">P3-F3A</td><td colspan="3">P3-F3B</td></tr>
<tr><td>100</td><td>99.5</td><td>97.9</td><td>100</td><td>99</td><td>98.8</td></tr>
</table>
[a] Live birth index = number of pups born alive/number of born pups x 100.
</td><td></td></tr>
<tr><td>Reproductive assessment by continuous breeding<br><br>Performed</td><td>Test material: boric acid<br><br>Purity: >99%<br><br><u>Doses/conc</u>.: 0, 1000 ppm, 4500</td><td>Maternal effects<br>Statistically significantly decreased body weight (data not shown) in the females of the high dose group (221 mg B/kg bw/day).<br><br>Effects on the offspring<br><u>1000 ppm (equivalent to 26.6 mg B/kg/day):</u></td><td>Fail et al. 1991</td></tr>
</table>

| **Method, guideline, deviations if any, species, strain, sex, no/group[18]** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| according to the NTP's Reproductive Assessment by Continuous Breeding Protocol<br><br>Mouse (Swiss) male/female<br><br>n = 19/sex/dose groups<br><br>No litters were born to F0 parents exposed to 9000 ppm, and only three litters were born alive to the 4500 ppm breeding pairs after cohabitation ended. Thus, F1 animals in the control and 1000 ppm groups were chosen for assessing the F1 generation.<br><br>Reliability: 2 (reliable with restrictions) | ppm or 9000 ppm equivalent to 0, 152, 636 and 1262 mg boric acid/kg bw/day, equivalent to 0, 26.6, 111.3 and 221 mg B/kg bw/day, respectively.<br><br>Exposure: 27 weeks (daily in feed) | F1 pups: no statistically significant changes were observed.<br>F2 pups: statistically significantly ($p<0.05$) decreased adjusted live pup weight (by approx. 3% compared to control).<br><br>4500 ppm (equivalent to 111.3 mg B/kg/day):<br>F1 pups: statistically significant decreased parameters compared to controls:<br>- adjusted live pup weight by approx. 14%;<br>- number of litters/pair by approx. 51%;<br>- live birth index by approx. 11%.<br><br>Only 1/19 F1 dams had 5 litters and all her pups in the 4th litter were born dead.<br><br>9000 ppm (equivalent to 221 mg B/kg/day):<br>F0: No litters were born to F0 animals. | |
| **Assessment of embryonic or foetal death after treatment of male rats during spermatogenesis**<br><br>No guideline specified<br><br>Rats (white outbred), male<br><br>n = 6 males/dose group<br><br>Males were administered test substance during the entire | Test material: boric acid<br><br>Purity: unknown<br><br>Doses/conc.: 0, 1 and 10 mg B/kg bw/day<br><br>Exposure: 60 days, daily oral gavage | **1 mg B/kg bw/day**<br>Statistical significant ($p\leq0.05$) changes compared to control were observed for the following parameters:<br>- living embryos/female: 8 ± 0.62 (controls: 9.71 ± 0.33);<br>- dead embryos/female: 1.3 ± 0.35 (controls: 0.714 ± 0.45);<br>- post-implantation loss: 13.62 ± 5.1 % (controls: 6.92 ± 1.67).<br><br>**10 mg B/kg bw/day**<br>Statistically significant ($p\leq0.05$) changes compared to controls were observed for the following parameters:<br>- living embryos/female: 6 ± 0.61 (controls: 9.71 ± 0.33);<br>- dead embryos/female: 1.3 ± 0.25 (controls: 0.714 ± 0.45);<br>- post-implantation loss: 18.0 ± 6.1 % (controls: 6.92 ± 1.67). | Marat et al. 2018 |

| **Method, guideline, deviations if any, species, strain, sex, no/group[18]** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| spermatogenesis cycle. At the end of the exposure period, the males were mated with untreated females at a 1:1 ratio. Gestation was terminated at day 20 and the number of implantation sites, resorptions, and embryos on the uterine horns and the corpus luteum count in the ovaries were investigated**.** | | | |
| *Barium chloride* | | | |
| **Reproductive and fertility assessment**<br><br>No guideline specified<br><br>Rat (Fischer 344) male/female<br><br>Mice (B6C3F1) male/female<br><br>n = 20/sex/dose group/species<br><br>Differences when comparing to OECD TG 421: dosing only prior to mating, no individual animal data/tables provided, histopathologic examination, data on food consumption only provided for core study animals, no humidity, sex of pups, and data on stability of test substance in | Test material: barium chloride dihydrate<br><br>Purity: 99.5%<br><br><u>Rats</u>:<br><u>Doses/conc.:</u> 0, 1000, 2000, and 4000 ppm barium chloride dehydrate, equivalent to 0, 120, 240 and 480 mg/kg bw/day, respectively<br><br><u>Mice</u>:<br><u>Doses/conc.:</u> 0, 500, 1000 and 2000 ppm, equivalent to 0, 90, 180 and 360 mg/kg bw/day, respectively<br><br><u>Exposure</u>:<br>The males were exposed prior to mating, for 60 days, and the females for 30 days, daily in drinking water. | **Observed effects in rats**<br>*Maternal effects*:<br>At 480 mg/kg bw/day, one dam died during the last week of the treatment, the necropsy revealing 7 foetuses and one resorption site. No information is presented on the maternal body weight or on signs of general toxicity.<br><br>*Embryo/foetal effects*:<br>The live pup weight at birth was statistically significantly ($p<0.01$) reduced (5.20 ± 0.06 g vs. 5.70 ± 0.09 g in controls).<br>The average litter size on postpartum day 5 was reduced compared to controls (7.1 ± 0.56 vs. 9.3 ± 1.16 pups in controls). Pup survival until postnatal day 5 was >99% in all treated groups and controls (data not shown).<br>No external abnormalities were observed in the offspring.<br><br>**Observed effects in mice**:<br>*Maternal effects*:<br>There was no evidence of maternal toxicity in the treated mice: maternal weight gain during pregnancy was comparable to controls for all dose groups (data not shown).<br><br>*Embryo/foetal effects*:<br>At the 180 mg/kg bw/day, a statistically significant ($p<0.05$) reduction in the average litter size was observed on postnatal day 0 (7.9 ± 1.02 pups vs. 10.7 ± 0.40 pups in the control group) and postnatal day 5 (7.7 ± 0.97 pups vs. 10.8 ± 0.38 pups in the control group).<br>A few pups (number not reported) were found dead at birth for all dose levels (not specified for controls), and survival from postnatal day 0 to postnatal day 5 ranged between 98 – 100 % (dose level not specified, data not shown).<br>No statistical differences in live pup body weights and no | Dietz et al. 1992 |

| **Method, guideline, deviations if any, species, strain, sex, no/group[18]** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| vehicle given. Only the average results of the controls and the high dose groups of each species are available.<br><br>The reproductive and fertility assessment was performed on separate groups of rats and mice than the ones used for the sub-chronic toxicity study. | | external anomalies were seen in the offspring. | |
| **Prenatal developmental toxicity study**<br><br>Performed according to OECD TG 414 and GLP guidelines<br><br>Rat (Wistar), female<br><br>n = 24 rats/dose group<br><br>Reliability: 1 (reliable without restrictions) key study[20] | Test material: barium chloride dihydrate<br><br>0, 10, 30 and 100 mg/kg bw<br><br>Exposure:<br>GD 0 – 20, (oral gavage, daily) | **NOAEL (maternal toxicity)**: 30 mg/kg body weight<br>**NOAEL (prenatal developmental toxicity)**: ≥ 100 mg/kg body weight<br><br>**Maternal effects**:<br>Two dams from the 100 mg/kg bw group died on the last day of treatment. Another dam showed conditional decline until last day of treatment, the necropsy showing hydrothorax, haemorrhages in the liver and haemorrhagic discharge in the vagina.<br>A slightly, but statistically significantly reduced body weight gain was observed in the high dose group as compared to the control group during the first three days of dosing (data not shown).<br><br>**Embryo/foetal effects**:<br>The mean foetus weight was comparable in all dose groups (data not shown). Foetal external, visceral and skeletal examinations did not reveal any treatment-related effects.<br>At 100 mg/kg bw all foetuses of the 3 dams that died and/or showed severe clinical signs of toxicity were dead. | Study report, 2014[21] |

Table 21: Summary table of human data on adverse effects on development

| **Type of data/report** | **Test substance** | **Relevant information about the study (as applicable)** | **Observations** | **Reference** |
|---|---|---|---|---|
| *Boric acid and borate salts* | | | | |
| | | | | |

[20] The reliability score for this study is according to the publically disseminated REACH Registration dossier of barium chloride, available at https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15037/7/9/3

[21] As presented in the publically disseminated REACH registration dossier for barium chloride, available at https://www.echa.europa.eu/web/guest/registration-dossier/-/registered-dossier/15037/7/9/3

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| **Study type: cohort study (retrospective)**<br><br>Questionnaire survey<br><br>Epidemiological studies on reproductive boron effects on women are scarce. The present study was designed to fill this gap, investigating possible boron-mediated developmental effects in women environmentally exposed in boron-rich areas. | The study investigated boron-environmental exposure of women residing near a borate-processing plant (Bandirma) and a boron-mining plant (Bigadic Boron Works), both located in Turkey. | HYPOTHESIS TESTED:<br>The global hypothesis was that the means of the three groups were equal (Kruskal-Wallis test).<br><br>METHOD OF DATA COLLECTION<br>Details: Demographic information and information on pregnancy outcomes were obtained by a questionnaire survey. Information on possible confounders (alcohol consumption, smoking, pesticide application) was also obtained.<br>This study did not include pregnant women, as pregnancy monitoring was not within the scope of the project.<br>All participating women, both in Badirma and in Bigadic, accepted to provide biological samples (blood and urine) and specimens of food from their meals (breakfast, lunch, dinner), as well as drinking water samples.<br><br>- Air sampling:<br>Air sampling was performed at two and five different sites of Bandirma and Bigadic, respectively. The sampling sites were representative of the appropriate study area. Static air sampling was performed using IOM samplers and personal air sampling pumps (SKC, AirCheck 2000). The flow rate was 2 L/min, and the sampling time was 8 h. SKC (GLA-5000), 5-µm and 25-mm filters, were used to sample inhalable dust.<br><br>- Biological sampling:<br>**Bandirma**: sampling was performed on pre-scheduled dates in the guesthouse of | Bandirma: Although significant boron exposure occurs in employees of the local boric acid production plant and the commercial port of Bandirma, environmental boron exposure is negligible for the general population living in Bandirma.<br><br>Bigadic: Boron concentrations in the drinking water (environmentally) of Iskele were very high, i.e. around 12.2 mg B/L.<br><br>Boron concentrations in air samples taken from Bandirma and Bigadic were lower than the limit of quantitation (LOQ). Therefore, environmental boron exposure by inhalation was not taken into account when estimating DBE levels. The major and relevant sources of boron exposure, in both Bandirma and Bigadic, were drinking water and food. The daily drinking water consumption of all participating females was assumed to be 2 L/day.<br>The daily boron exposure via food was estimated using the "double plate method" for both lunch and dinner (i.e. the provided food samples from lunch and dinner menus were equal to the amounts actually consumed). Local bread, cheese, eggs and olives were mostly consumed for breakfast. Boron concentration in these food samples was negligible. Therefore, boron exposure via breakfast was neglected.<br><br>DBE levels (mg B/day, Mean ± SD (range)):<br>Low exposure group: 9.73 ± 5.29 (2.26–38.27);<br>Medium exposure group: 21.62 ± 7.87 (8.08–39.71);<br>High exposure group: 24.67 ± 11.39 (10.47–57.86).<br><br>Blood boron levels (ng B/g | Duydu et al. 2018b |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| | | Eti Mine Works General Management that is located distant from both the local boric acid production plant and the commercial port. The participants were asked to bring samples of their actual meals (breakfast, lunch, dinner) and of their drinking water. Containers for food and water were provided. After completing the questionnaire, blood and urine samples were taken and stored.<br><br>**Bigadic**: sampling was performed by visiting the participants at home. Again, after completing the questionnaire, blood, urine, drinking water and meal samples (breakfast, lunch and dinner) were stored.<br><br>Vein blood samples were drawn into Vacutainer collection tubes containing heparin and stored at 4 °C for subsequent boron determination. Spot urine samples of all volunteers were collected in polypropylene containers and kept at − 20 °C until analysis of boron and creatinine. Creatinine analysis was performed using the creatinine assay kit of Cayman Chem. Corp. Drinking water and food samples were stored in polypropylene containers at -20 °C until boron analysis.<br><br>STUDY PERIOD:<br>2014 – 2017<br><br>STUDY POPULATION<br><u>- Total population</u>: 199 women residing near Bandirma and Bigadic, divided into 3 different groups based on the measured blood boron levels, as follows: | <u>blood, Mean ± SD (range)):</u><br>Low exposure group: 39.74 ± 27.60 (3.28–99.28);<br>Medium exposure group: 124.19 ± 13.10 (100.35–1496.74);<br>High exposure group: 274.58 ± 213.00 (151.81–975.66).<br><br>The study covered a number of 199 women who altogether gave birth to 326 children (i.e. 162 girls and 164 boys), with the following measured parameters:<br><br><u>Number of childless women:</u><br>Low exposure group: 14;<br>Medium exposure group: 1;<br>High exposure group: 0.<br><br><u>Number of low body weight children (<2500 g):</u><br>Low exposure group: 21;<br>Medium exposure group: 6;<br>High exposure group: 7.<br><br><u>Number of very low body weight children(<1500g):</u><br>Low exposure group: 2;<br>Medium exposure group: 1;<br>High exposure group: 1.<br><br><u>Number of preterm births:</u><br>Low exposure group: 12;<br>Medium exposure group: 1;<br>High exposure group: 4.<br><br><u>Number of children with congenital anomalies:</u><br>Low exposure group: 6;<br>Medium exposure group: 1;<br>High exposure group: 1.<br><br><u>Number of spontaneous abortions (miscarriages):</u><br>Low exposure group: 21;<br>Medium exposure group: 6;<br>High exposure group: 6.<br><br><u>Number of stillbirths:</u><br>Low exposure group: 0;<br>Medium exposure group: 1;<br>High exposure group: 1.<br><br><u>Number of infant deaths:</u><br>Low exposure group: 2; | |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| | | -Low exposure group: blood boron concentrations < 100 ng B/g blood were (*n* = 143);<br>-Medium exposure group: with blood boron concentrations between 100 – 150 ng B/g blood (n = 29);<br>-High exposure group: with blood boron concentrations between >150 ng B/g blood (n = 27).<br><br>- Age of the study population (mean ± SD (range)):<br>Low exposure group (*n* = 143): 32.31 ± 6.77 (17–49);<br>Medium exposure group (*n* =29): 36.28 ± 6.95 (23–49);<br>High exposure group (*n* = 27): 34.56 ± 6.10 (24–46).<br><br>MEASURED PARAMETERS:<br>-DBE (daily boron exposure), boron concentrations in biological fluids (i.e. blood, urine), preterm births, numbers of children, birth weights of newborns, congenital anomalies, abortions, miscarriage, stillbirth, early neonatal death, neonatal death and infant death. | Medium exposure group: 2;<br>High exposure group: 0.<br><br>Birth weight of newborns (g, Mean ± SD (range)):<br>Low exposure group: 3213 ± 561 (1140 - 5000);<br>Medium exposure group: 3083 ± 563 (1400 - 4200);<br>High exposure group: 3112 ± 709 (1200 - 4750).<br><br>Birth weight of newborns - girls (g, Mean ± SD (range)):<br>Low exposure group: 3154 ± 536 (1140 - 4250);<br>Medium exposure group: 2991 ± 615 (1400 - 4000);<br>High exposure group: 3057 ± 674 (2000 - 4000).<br><br>Birth weight of newborns – boys (g, Mean ± SD (range)):<br>Low exposure group: 3269 ± 580 (1400 - 5000);<br>Medium exposure group: 3209 ± 464 (2000 - 4200);<br>High exposure group: 3142 ± 745 (1200 - 4750).<br><br>Birth weights of newborns (girls, boys, girls + boys) were statistically not different between low, medium and high exposure groups ($p < 0.05$).<br>The boron-mediated effects on the birth weights analysed using linear spline regression models with two knots at 100 and 150 ng B/g blood, did not show any statistically significant associations.<br>The numbers of newborns with LBW and VLBW were also compared between the low, medium and high exposure groups, and no statistically significant differences were reported.<br><br>Conclusions:<br>Based upon the presented results, the authors concluded that environmental exposure to boron does not have an adverse effect on the development of the offspring. | |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| **Mother-child cohort study (prospective)**<br><br>Endpoint addressed: foetal development<br><br>Interviews<br><br>The study was designed to see the pregnant women at least once during pregnancy; preferably 2–3 times (once per trimester) in order to obtain repeated measures of exposure. | Boron environmental exposure of pregnant women residing in northern Argentina. | METHOD OF DATA COLLECTION<br>Details: interviews conducted by the authors. At enrolment, the women were interviewed regarding family characteristics, including known diseases, preferred diet, last menstrual period (LMP), and pre-pregnancy weight. Data on maternal age, parity (number of born children), parental monthly income, years of maternal education, smoking, alcohol consumption, chewing of coca leaves, and prenatal vitamin supplementation were collected at the follow-up visits.<br><br>Biological samples:<br>-Serum samples were fractionated from whole blood samples collected in Trace Elements Serum Clot Activator tubes (Vacuette®, Greiner bio-one, Kremsmunster, Austria).<br>-Spot urine samples were collected in disposable trace element-free plastic cups and transferred to 20 mL polyethylene bottles (Zinsser Analytic GMBH, Frankfurt, Germany). Blood and spot-urine samples were collected at each visit, at which time the women were also interviewed about encountered health problems.<br><br>Waters samples: were repeatedly collected during the study period using 20-mL polyethylene bottles.<br><br>Boron concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700×, Agilent Technologies, Tokyo, Japan). | **Parameters measured in the mothers:**<br>-Average pre-pregnancy weight: 55 kg (range 38–86 kg);<br>-Average height: 153 cm (range 134–169 cm);<br>-BMI: 24% had a BMI above 25;<br>-Time of residing in the area: 96% had lived in the study area for several years (mean time 18 years, range 0.1–40 years);<br>-Median boron levels in drinking water (µg/L)<br>-tertile 1 (n=60): 5246;<br>-tertile 2 (n=60): 5965;<br>-tertile 3 (n=60): 6072;<br><br>-Median serum boron levels (µg/L) (range):<br>-first trimester (n=31): 118 (32-232);<br>-second trimester (n=99): 131 (20-273);<br>-third trimester (n=152): 135 (26-315);<br><br>-Whole blood boron levels (µg/L) (range):<br>-first trimester (n=31): 131 (55-245);<br>-second trimester (n=99): 119 (38-210);<br>-third trimester (n=152): 139 (47-280);<br><br>-Urinary boron levels (µg/L) (range):<br>-first trimester (n=31): 10 076 (3107-19681);<br>-second trimester (n=99): 9881 (2803-23058);<br>-third trimester (n=152): 10 307 (2972-21144);<br><br>-Whole blood lithium levels (µg/L) (range):<br>-first trimester (n=31): 21 (6.6-54);<br>-second trimester (n=99): 23 (4.1-52);<br>-third trimester (n=152): 26 (5.7-63);<br><br>-Urinary lithium levels (µg/L) | Igra et al. 2016 |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| | | Because arsenic, cesium and lithium were also present at elevated concentrations in the drinking water and these elements may also impair foetal growth, the exposure to these was considered. We additionally measures lead, cadmium and selenium to test for confounding. Cesium and lithium were measured in whole blood and urine, lead and cadmium in whole blood and selenium in serum, all by ICP-MS. Exposure to arsenic was assessed by the sum concentrations of inorganic arsenic (iAs) and its mono- and dimethylated metabolites (MMA and DMA) in urine, measured using HPLC-HG-ICPMS.<br><br>STUDY PERIOD:<br>2012 – 2013<br><br>STUDY POPULATION<br>- Total population: 180 women (out of the initial 194 women enrolled in the study, 182 were interviewed and provided samples and 2/182 had miscarriages).<br><br>- Age of the study population: average 25 years old (13 – 41 years)<br><br>MEASURED PARAMETERS:<br>-water boron levels, boron concentrations in biological fluids (i.e. blood, serum and urine);<br>-pregnancy outcomes: birthweight (g), length (cm) and head circumference (cm), (measured by the health care personnel immediately after birth (for most women) or after a few hours for seven women | (range):<br>-first trimester (n=31): 1117 (209-3768);<br>-second trimester (n=99): 1398 (262-3509);<br>-third trimester (n=152): 1465 (273-3732);<br><br>-Whole blood cesium levels (µg/L) (range):<br>-first trimester (n=31): 132 (12-288);<br>-second trimester (n=99): 107 (8.3-220);<br>-third trimester (n=152): 111 (8.9-253);<br><br>-Urinary arsenic levels (µg/L) (range):<br>-first trimester (n=31): 98 (31-458);<br>-second trimester (n=99): 104(26-282);<br>-third trimester (n=152): 129 (33-414);<br><br>**Pregnancy outcomes**:<br>-Mean birth weight: 3022 ± 459 g (range 1250–4500 g), with 9.4% weighing < 2500 g.<br>-Average birth length: 48 ± 2.3 cm (range 39–53 cm)<br>-Average head circumference: 34±1.7 cm (range 26–40cm)<br>-Average gestational age at birth: 39 weeks (range 29–42 weeks), and 18% of the infants were born pre-term (before 37 gestational weeks).<br><br>The adjusted mixed effect linear models showed that the serum boron concentration increased by 3.1 µg/L per gestational week on average (95% CI 1.9; 4.4, p-value < 0.001). The effect estimate for the inverse association between serum boron concentrations (above 80 µg/L) and birth length increased by 28% when considering only the third trimester instead of the whole pregnancy (B −0.088 for each 10 µg/L increase in serum boron concentration, 95% CI −0.14; −0.036, p-value = | |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| | | (3.9%) who delivered at home). | 0.001). The inverse association between serum boron concentrations (above 80 µg/L) and birth weight was statistically significant, and the fully adjusted effect estimate increased >2.5 times (from −4.5 to −12 g per 10 µg/L increase in serum boron) when considering only exposure in the third trimester. No statistically significant associations between serum boron concentrations > 80 µg/L and birth head circumference was found in any model.<br><br><u>Conclusions:</u><br>The results of this study show that elevated environmental boron levels have a statistically significant effect on the birth size of newborn. | |
| **Cohort study (retrospective)**<br><br>Endpoint addressed: toxicity to reproduction<br><br>Criteria for selection was the presence of legal marriage regardless of whether one member was dead or whether there had been a divorce. The study was carried out by home visits. Workers and other related individuals were contacted at borate plants and pits.<br><br>Questionnaires were arranged in order to obtain | The study investigated boron-environmental exposure of residents from villages located near the borate-processing plant Bigadic, Balikesir county, Turkey. | <u>Details on study design</u>:<br>The study population was divided into three sub-groups. The individuals that were interviewed in each subgroup served as probands for the study. The first subgroup of probands was identified in Region 1 which covers an area near boron-rich territories. Dwellings of Region 1 were located close to borate pits and a processing plant. Region 2 probands were from villages far from boron deposits, but were within the same zone. Region 3 probands were born and lived in areas with a mixed group, some near to and some far from deposits and pits. In Region 1, drinking water forming from (natural) springs and wells contained 29 ppm boron, but in Region 2 the concentration was between 0.3 and 0.50 ppm. In the third region, no measurements were regularly made but boron | The infant death rate in Region 2 (low boron area) was higher than those of other regions (significantly different).<br><br>Although difficult to recognise spontaneous abortions and stillbirths in a retrospective study based solely on the description of the probands (mostly females), these were considered separately, but no differences were found.<br><br>The observed number of congenital malformation was not sufficient within the study groups to perform statistical tests. | Tuccar et al. 1998 |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| the number of pregnancies, early infant deaths, congenital malformations, stillbirths and spontaneous abortions. | | content was not known to be too high. In all three areas there were active and former borate workers. From Region 1, 226 families over three generations with respect to probands (that of the proband being the second) and from Region 2, 164 families were included. There were 177 families from Region 3 and 80 from Kirka. | | |
| **Retrospective study**<br><br>Endpoint addressed: developmental toxicity / teratogenicity.<br><br>This study was based on interview data from a larger study of workplace exposure to boron-containing compounds and adverse male reproductive effects. The reproductive effects data were obtained by self-report of delays in pregnancy, pregnancy outcomes, total number of children, and gender of children. | The study investigated occupational boron-exposure of workers from boron mines and processing plants located in the city of Kuandian, China. | The authors evaluated reproductive health in a cohort of boron mining and processing male workers (N=936) and a comparison group of males (N=251) in northeast China.<br><br>The comparison group was selected from a community 30 miles away from the boron mines and processing plants with a known low background of environmental boron.<br><br>No exposure measurements were available for the wives of the workers whose boron exposure would be through environmental sources such as food and water. However, concentrations of boron in the surface water, well water, soil, legumes and potatoes of the boron workers group were greater than in the comparison group. | Exposure estimates for the boron workers was 31.3 mg boron/day and 1.40 mg B/day for the comparison group (Scialli et al. 2010).<br><br>Well water in the boron group ranged from 37 to 600 times the comparison group, and the mean boron concentrations in vegetables and potatoes from the boron group was approximately double those found in the comparison group.<br><br>Reproductive health parameters evaluated included: delay in pregnancy, multiple births, spontaneous miscarriage, induced abortion, stillbirth, tubal or ectopic pregnancy, and boy/girl ratio.<br><br>No statistically significant differences were observed between the boron workers and the comparison group after adjustment for age, educational level, race, smoking, ethanol use, and soybean intake. | Chang et al. 2006 |
| **Assessment of environmental daily boron exposure limits** | The study investigated environmental exposure to boron of residents of Balikesir area. | The aim of the study was to estimate daily boron exposure in 66 males in Turkey living in a B-rich area using water containing at least 2 mg/L boron with an average age of 38 - 55 (SE 1.66) years and an average number of years of | The average daily boron exposure was calculated as 6.77 (SE 0.47) mg in the study group and 1.26 (SE 0.1) mg in the controls. None of the subjects reported any health problems that may be linked to high boron exposure. | Korkmaz et al. 2007 |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| | | residence in the boron rich area of 35 - 89 (SE 1.73). Another group of 57 males living in the city centres of Balikesir and Ankara were taken as controls; the average age and number of years of residence for this group were 29.44 (SE 1.43) and 10.26 (SE 1.83) years respectively. As it is assumed that boron levels in urine reflect daily boron exposure, the amount of urinary boron of both the study and control groups was analysed using an inductively coupled plasma optical emission spectrometry technique (ICP-OES). | | |
| **Case control study (retrospective)**<br><br>Endpoint addressed: developmental toxicity / teratogenicity<br><br>Supporting study | The study investigated the effect of vaginal tablets containing boric acid. | The effects of the use of boric acid vaginal tablets for treatment of infectious diseases of the genital organs were evaluated in a Hungarian Case Control Surveillance of Congenital Abnormalities (HCCSCA) study.<br><br>In most cases, treatment consisted of two vaginal tablets of 30 mg each daily for 7 days. | For the 22843 infants born with congenital abnormalities in the study group, 43 mothers (0.19 %) had received boric acid treatment and for the 38151 controls, 52 mothers (0.14 %) had received boric acid treatment.<br><br>There were no significant differences between the groups in maternal sociodemographic characteristics, occurrence of acute and chronic diseases and frequently used drugs. The extremely high prevalence of acute infectious diseases of the genital organs (85.8 % in the study group and 91.9 % among controls) explains the use of the boric acid.<br><br>Cases of congenital abnormalities affecting the skeletal system only occurred in the offspring of others who were treated with boric acid during their entire pregnancy. In this study there was a higher risk of neural tube defects when boric acid was used during the second and third months of pregnancy, but this finding was based on only two cases. | Acs et al. 2006 |

| Type of data/report | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| | | | It is suggested that topical exposure to boric acid is unlikely to induce developmental toxicity because unless the skin or vaginal epithelium is severely damaged, boric acid absorption is limited. | |

Table 22: Summary table of other studies relevant for developmental toxicity

| Type of data/report[22] | Test substance | Relevant information about the study (as applicable) | Observations | Reference |
|---|---|---|---|---|
| *Boric acid* | | | | |
| **Benchmark dose (BMD) approach**<br><br>Rat (Sprague-Dawley)<br><br>Reliability: 2 (reliable with restrictions) | Test material: boric acid<br><br>Exposure: 20 days (oral: feed). | In this analysis of the developmental toxicity observed in rats exposed to boric acid in their diet, benchmark dose (BMD) analyses have been conducted using two existing studies. By considering various endpoints and modelling approaches for those endpoints, the best approach for incorporating all of the information available from the studies could be determined. In this case, the approach involved combining data from two studies which were similarly designed and were conducted in the same laboratory to calculate BMDs that were more accurate and more precise than from either study alone. | **BMD (developmental toxicity):** 59 mg/kg bw/day, equivalent to 10.3 mg B/kg bw/day, based on decreased foetal body weight provided the best basis for BMD calculations.<br><br>The benchmark dose is defined as the 95 % lower bound on the dose corresponding to a 5 % decrease in the mean fetal weight (BMDL05). Results are based on the studies of Heindel et al. 1992 and Price et al. 1996a,b. | Allen et al. 1996 |

[22] The reliability score of the study presented in Table 22 is according to the CLH dossier of boric acid, assessed by RAC in 2013.

### 10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

#### 10.10.5.1 Animal studies

**Data on barium diboron tetraoxide**

US EPA test guideline prenatal developmental toxicity study (Study report, 1993b)

In the prenatal developmental toxicity study performed according to US EPA guidelines and in compliance with GLP, 20 pregnant female rabbits were treated with 0, 2, 10, 20 mg barium diboron tetraoxide (equivalent to 0, 0.18, 0.9 and 1.8 mg B/kg bw/day, respectively) via oral gavage. The treatment was administered during gestation days (GD) 7 – 19 and observed until GD 29, when gross necropsy was carried out following sacrifice. At 1.8 mg B/kg bw/day, 1 dam died on GD 16 and another dam aborted on GD 22, and two other dams were found to be non-pregnant. No clinical signs attributable to the treatment were observed in the high dose-group dam that died on GD 16. No statistically significant changes in food consumption, body weight, mean body weight and gravid uterine weights were reported.

At the highest dose level, the necropsy examination of the foetuses revealed increased incidence (not clear if stat. sign.) of visceral malformations expressed as hydrocephaly in 12.5% of the litters (2/16 litters) and diaphragmatic hernia observed in one foetus (1/16 litters). However, due to the historical data showing hydrocephaly in control animals, this developmental effect was not considered treatment-related by the study director. Skeletal malformations, primarily expressed as fused sternebrae, extra ossification sites, vertebral anomalies with/without associated rib anomalies were seen in 5.6%, 0.78%, 4.2% and 2.91% of foetuses at 0, 0.18, 0.9 and 1.8 mg B/kg bw/day, respectively. At the highest dose level, 1/103 foetuses presented carpal flexure on the right front limb. However, it has to be noted that unusually high external, visceral and skeletal malformation incidences were reported for the control groups of this study.

According to the presented findings, the NOAEL for maternal toxicity in rabbits was 0.9 mg B/kg bw/day, while the NOAEL for developmental toxicity was set at 1.8 mg B/kg bw/day. No information was available from the necropsy examination performed on the foetuses of the high-dose group dam that died during the treatment. The dose-rationale of this study was based on a preliminary range-finding study where pregnant female rabbits were administered via oral gavage doses of 0, 20, 55, 90, 125 and 160 mg/kg bw/day (equivalent to 0, 1.8, 4.95, 8.1, 11.25 and 14.4 mg B/kg bw/day), during GD 7 – 19. One out of 7 dams died at 1.8 mg B/kg bw/day. Maternal toxicity, expressed as mortality and changes in the general clinical condition of the animals, was observed from 1.8 mg B/kg bw/day and higher. No information was available on the performed foetal examinations. No adverse effects on development were reported at the dose levels available for evaluation (i.e. 20, 55 and 90 mg/kg bw/day, equivalent to 1.8, 4.95 and 8.1 mg B/kg bw/day, respectively) (data not shown).

**Data on boric acid and borate salts**

The assessment of adverse effects on the development of the offspring of barium diboron tetraoxide is supported with read-across data from studies via oral exposure of boric acid and borate salts. In aqueous solutions at physiological and acidic pH, low concentrations of simple borates such as boric acid and borate salts will predominantly exist as undissociated boric acid. The toxicokinetics and toxicological effects of systemic barium diboron tetraoxide after oral exposure are therefore expected to be similar as boric acid and borate salts.

Prenatal developmental toxicity in rats (Price et al. 1996a)

Price et al. 1996a conducted a GLP-compliant study where female rats were administered 0, 19, 36, 55, 76 and 143 mg boric acid (equivalent to 0, 3.3, 6.3, 9.6, 13.3, 25 mg B/kg bw, respectively) via diet in two different phases: Phase I when teratologic evaluation was performed (days 0 – 20 post-mating) and Phase II for postnatal evaluation (the dams delivered and the pups were sacrificed after weaning). No maternal deaths occurred and no treatment-related clinical signs of general toxicity were observed in the dams, at any dose level. A statistically significant reduction in the mean foetal body

weight per litter was observed at the two highest dose levels (i.e. by approx. 6% at 13.3 mg B/kg bw/day and by approx. 13% at 25 mg B/kg bw/day, compared to controls). The viability of the offspring was not affected in any dose group. Treatment-related skeletal changes were observed at the highest dose levels. Thus, statistically significant increases in the incidence of short rib XIII (i.e. by approx. 1.5% at 13.3 mg B/kg bw/day and by approx. 3.4% at 25 mg B/kg bw/day, compared to controls) and wavy rib (by approx. 2.1 at 13.3 mg B/kg bw/day and by approx. 10% at 25 mg B/kg bw/day, compared to controls) amongst offspring were reported. Based on the observed results, the LOAEL for skeletal effects in rats was 13.3 mg B/kg bw/day and the NOAEL was 9.6 mg B/kg bw/day.

Moreover, the authors collected blood samples from the pregnant female rats used for Phase I investigation and prepared the samples for boron analysis through inductively coupled plasma optical emission spectrometry (Price et al. 1997). The average blood concentrations of boron increased with increasing dietary levels of boron, giving rise to 0.229 ± 0.143, 0.564 ± 0.211, 0.975 ± 0.261, 1.27 ± 0.298, 1.53 ± 0.546, or 2.82 ± 0.987 µg B/g whole blood for the control through all the dose levels, respectively. The maternal blood levels of boron were positively correlated with embryo/foetal toxicity. Dams exposed to 9.6 mg B/kg bw/day, had a level of 1.27 ± 0.298 µg B/g whole blood which corresponded with the NOAEL for developmental toxicity (9.6 mg B/kg bw/day). The developmental toxicity LOAEL (13.3 mg B/kg bw/day) corresponded to a blood boron concentration of 1.53 ± 0.546 µg B/g whole blood of the dams exposed to 76 mg boric acid/kg bw/day.

Prenatal developmental toxicity studies in rabbits (Price et al. 1996b; Heindel et al. 1994)

In two prenatal developmental toxicity studies, pregnant female rabbits were administered 0, 62.5, 125 and 250 mg/kg bw/day boric acid (equivalent to 0, 11, 22 and 44 mg B/kg bw/day) via oral gavage during GD 6 – 19. Increased incidence of vaginal bleeding, considered to be treatment-related (2 – 11 pregnant females/day bled between GD 19 – 30), was observed at the highest dose level 44 mg B/kg bw/day. All does with vaginal bleeding had no live foetuses on GD 30. Reduced food intake and body weight gain were reported at the highest dose level (statistically significantly reduced by approx. 31% and 10%, respectively, as compared to controls) during the treatment period. However, the corrected (for gravid uterus weight) maternal weight change was increased.

At 44 mg B/kg bw/day statistically significant increased rate of resorptions per litter was reported (89.9% versus 6.3 in control, $p<0.05$) and 73% of the does had 100% resorptions. Consequently, the average number of live foetuses per litter in this dose group was severely reduced (2.3 compared to 8.8 in control, $p<0.05$).

The incidence of external malformations was also statistically significantly increased in the 44 mg B/kg bw/day dose group compared to controls (11.1% versus 0.8%, $p<0.05$).

Furthermore, statistically significantly increased incidences of visceral malformations were observed only at the highest dose level, i.e. interventricular cardiovascular septal defect (0.6% in controls vs. 57% at 44 mg B/kg bw/day), enlarged aorta (0% in controls vs. 36% at 44 mg B/kg bw/day), papillary muscle malformations (3% in controls vs. 14% at 43.5 mg B/kg bw/day) and double outlet right ventricle (0% in controls vs. 14% at 44 mg B/kg bw/day). Other visceral effects were agenesis of the gall bladder, enlarged gall bladder and enlarged heart. Based on the results reported by this study, the LOAELs for both maternal and developmental toxic effects were set at 44 mg B/kg bw/day.

It is also noted that the incidence of skeletal malformations was increased at 44 mg B/kg bw/day, although not statistically significant compared to control due to high background incidence of cleft sternum in the controls. The findings of increased incidences of fused ribs and fused sternebrae (7% versus 1.3% in control, and 7% versus 0% in control) at 44 mg B/kg bw/day (each effect seen in only 1 foetus, in separate litters) were also considered equivocal.

The studies performed in rats and rabbits by Price and colleagues (1996a and b) show that boron treatment led to maternal toxicity only for the female rabbits and adverse effects on the development of both rabbit and rat offspring, mainly expressed as visceral and skeletal malformations. Moreover, the developmental effects in rats were observed in the absence of maternal general toxicity and are thus considered relevant for classification purposes.

Prenatal developmental toxicity study in rat and mouse (Heindel et al, 1992)

Heindel et al. 1992 investigated the developmental toxicity of boric acid in both rat and mouse pregnant females. Rats were administered 0, 78, 163 and 330 mg/kg bw boric acid (equivalent to 0, 14, 29 and 58 mg B/kg bw) via feed during GD 0 – 20 and 539 mg boric (equivalent to 94 mg B/kg bw) acid during GD 6 – 15. In rats, at 29 and 58 mf B/kg bw/day, maternal toxicity was reported as kidney lesions in mice and increased liver and kidney weights for both species. In mice, at the highest dose level (175 mg B/kg bw/day) statistically significantly reduced body weight gain (by approx. 25%) of the dams was also observed. However, when correcting for gravid uterus weight, there was no statistically significant difference compared to control.

In the rat, developmental toxic effects such as statistically significantly decreased average foetal body weight for all treated groups ranging from 7% decrease (at 14 mg B/kg bw) to 50 % (at 94 mg B/kg bw), malformations of the central nervous system (i.e. enlarged lateral ventricles of the brain) in 5.5% of the foetuses at 58 mg B/kg bw/day and 26.5% of the foetuses at 94 mg B/kg bw/day, eyes (i.e. displaced eyes, convoluted retina) in 11% of the foetuses at 94 mg B/kg bw/day, were observed. Moreover, increased incidences of skeletal malformations such as agenesis of rib XIII in 6.2% and 12.5% of foetuses (compared to 0.23 and 0% in the respective control groups) at 58 and 94 mg B/kg bw/day, respectively, were reported. Shortening of rib XIII was also seen in 39% and 37% of foetuses, at 58 and 94 mg B/kg bw/day, respectively. Cardiovascular and central nervous system morphological defects were absent in mice foetuses. A statistically significantly increased resorption rate was reported at 175 mg B/kg bw/day (approx. 19% per litter vs. 6% in controls). Furthermore, statistically significantly reduced foetal body weight by approx. 12% at 79 mg B/kg bw/day and by approx. 33% at 175 mg B/kg bw/day, and an increased incidence of short rib XIII (4% vs. 0% in controls) at the highest dose level, were observed. Based on the findings of this study, the LOAEL for developmental toxicity in rats was 14 mg B/kg bw/day while the LOAEL for developmental effects in mice was 79 mg B/kg bw/day. The results of this study showed that rats had a greater sensitivity to the developmental effects of boric acid than mice.

Multi-generational reproduction toxicity studies in rat (Weir and Fisher 1972) and mouse (Fail et al., 1991)

The three-generation study performed by Weir and Fisher 1972 in rats showed that live birth indices, litter size, weights and external appearance of the offspring for all filial generations (F1, F2 and F3) at both 5.9 and 17.5 mg B/kg bw/day, were comparable with those of the control groups. No information on the developmental effects of boric acid or borax was available at 58.5 mg B/kg bw/day because the parents of the highest dose group were sterile. Furthermore, in a multi-generation study in mice, the lowest dose level (26.6 mg B/kg bw/day) revealed statistically significantly decreased live pup weight (by approx. 3% as compared to controls) in the pups of the F2 generation. At the same dose level, there were no statistically significant changes from controls on pup body weights of the F1 generation (Fail et al. 1991). Statistically significantly decreased live birth index (by approx. 11% vs. controls) and number of litters per pair (by approx. 51% vs. controls) were reported at the mid dose level (111.3 mg B/kg bw/day) for the F1 generation. None of the parental pairs produced any offspring at the highest dose level (221 mg B/kg bw/day).

Rodent dominant lethal test (Marat et al. 2018)

In a recent study, male rats were administered 0, 1 and 10 mg B/kg bw/day via oral gavage for 60 days and mated with untreated females after the cessation of the treatment (Marat et al. 2018). While a 94% increase in post-implantation loss and 82% increase in the number of dead embryos per female were reported at 1 mg B/kg bw/day, the post-implantation loss index increased by 157% at 10 mg B/kg bw/day.

Benchmark dose approach (Allen et al. 1996)

The summary table of other studies relevant for developmental toxicity (Table 12) presents a benchmark dose (BMD) approach performed by Allen et al. 1996. A BMD was developed based on the studies described above (Heindel et al. 1992; Price et al. 1996a) as an alternative for reference value calculations, instead of only using the NOAEL, since the BMD can be applied in a consistent

manner throughout different studies and is not limited to one of the experimental doses. The authors considered decreased foetal body weight as the most suitable basis for calculating the BMD of 59 mg boric acid/kg bw/day (10.3 mg B/kg bw/day), which is very close to the NOAEL value for developmental toxicity (55 mg boric acid/kg bw, equivalent to 9.6 mg B/kg bw) set by Price et al. 1996a.

***Conclusion on animal studies of barium diboron tetraoxide, boric acid and borate salts***

There are no clear findings of barium diboron tetraoxide developmental toxicity in rabbit offspring. The reported developmental effect (i.e. hydrocephaly) seen at 1.8 mg B/kg bw/day was not considered treatment-related by the study director, due to an increased incidence of hydrocephaly seen in the historical control data of the testing facility. Non-statistically significant incidences of other developmental effects (i.e. external and skeletal malformations) have been reported, but due to the control animals showing unusually high incidences of such effects, a clear conclusion regarding the developmental toxicity of barium diboron tetraoxide cannot be made solely based upon this study. Furthermore, the preceding dose-range finding study reported maternal toxicity expressed as mortality from 1.8 mg B/kg bw and higher. However, no information on the maternal (or foetal) examinations was available for this study. Since the dam that died in the PNDT study was internally normal and did not present any treatment-related clinical signs, it is thus not clear if maternal mortality was due to general toxicity or a gavage error.

Moreover, in a boric acid PNDT study performed in rabbits, no maternal deaths occurred and the LOAEL for maternal toxicity (based on reduced body weight gain and abortions) was the same as the LOAEL for developmental toxicity (based on increased resorptions and cardiovascular malformations), i.e. 43.5 mg B/kg bw/day (Price et al. 1996b). Given that the doses administered in the barium diboron tetraoxide PNDT study are considerably lower than those used in the PNDT study with boric acid, it is therefore not possible to conclude that barium diboron tetraoxide does not affect the development of the offspring.

According to Annex I, paragraph 3.7.1.4 of the CLP Regulation, *developmental toxicity primarily consists of the following major manifestations: (1) death of the developing organism, (2) structural abnormality, (3) altered growth and (4) functional deficiency*. The above presented animal data on boric acid and borate salts show clear evidence of boron developmental effects in different species, i.e. rats, mice and rabbits, as follows:

1) Death of the developing organism

In a continuous breeding study in mice, statistically significantly decreased live birth index (by approx. 11% vs. controls) and number of litters per pair (by approx. 51% vs. controls) were observed at 111.3 mg B/kg bw/day (Fail et al. 1991). In rabbits, markedly increased rates of resorptions per litter (89.9 %) where only 6 litters survived until GD 30 (compared to 18 – 23 litters in controls) were seen in the presence of some maternal toxicity at 44 mg B/kg bw/day (Price et al. 1996b; Heindel et al. 1994). Moreover, in rats at 94 mg B/kg bw/day (Heindel et al. 1992) the rate of resorptions was also increased (36% resorptions per litter vs. 4% in controls) at the highest dose tested (94 mg B/kg bw/day).

2) Structural abnormality

In rats, skeletal malformations such as agenesis of rib XIII in 6.2% and 12.5% of foetuses and shortening of rib XIII in 39% and 37% of foetuses, at 58 and 94 mg B/kg bw/day, respectively, were seen in the absence of maternal toxicity (Heindel et al. 1992). Increased incidence of short rib XIII (i.e. by approx. 1.5% at 13.3 mg B/kg bw/day and by approx. 3.4% at 25 mg B/kg bw/day, compared to controls) in absence of maternal toxicity was also observed in the study by Price et al. (1996a). Similarly, in mice, significantly increased incidence of short rib XIII (4% vs. 0% in controls) was reported at 175 mg B/kg bw/day, in the absence of maternal toxicity.

Moreover, visceral malformations such as enlarged lateral ventricles of the brain in 5.5% of foetuses at 58 mg B/kg bw/day and 26.5% of the foetuses at 94 mg B/kg bw/day, as well as malformations of the eyes (i.e. displaced eyes, convoluted retina) in 11% of the foetuses at 94 mg B/kg bw/day, were also observed in rat (Heindel et al. 1992). While skeletal malformations were seen in both rat and mice

pups, the effects on the CNS and eyes were reported only for rats.

In rabbits, cardiovascular malformations such as interventricular septal defects (57% vs. 0.6% in controls), enlarged aorta (36% vs. 0% in controls), papillary muscle malformations (14% vs. 3% in controls) and double outlet right ventricle (14% vs. 0% in controls) were seen at the highest dose level (43.5 mg B/kg bw/day) where some maternal toxicity was also present (Price et al. 1996b). The incidence of skeletal defects (i.e. cleft sternum, detached extra rib – lumbar 1, fused sternebrae and fused rib) was increased for all dose levels (11, 22 and 44 mg B/kg bw/day), but not statistically significantly different from controls. As presented above, the effects on the skeletal system were consistently observed in rats, mice and rabbits while the cardiovascular defects were specific only for the rabbit offspring.

3) Altered growth

Markedly reduced ($p<0.05$) mean foetal body weights per litter were observed in rat pups, i.e. by approx. 6% at 13.3 mg B/kg bw/day and 13% at 25 mg B/kg bw/day, compared to controls, in the absence of maternal toxicity (Price et al. 1996a). Moreover, a severely dose-dependent decrease in average rat pup foetal body weight as compared to controls was noted for all dose levels (7, 13, 37 and 50% at 14, 29, 58 and 94 mg B/kg bw/day, respectively) where no marked maternal toxicity was evident.

Moreover, a significant decrease ($p<0.05$) in mouse foetal body weight was reported at 79 and 175 mg B/kg bw/day, where some maternal toxicity (effects on the kidneys) was observed only at the highest dose level (Heindel et al. 1992).

4) Functional deficiency

The CNS morphological defects (i.e. enlarged lateral ventricles of the brain) were seen in rats at 58 and 94 mg B/kg bw/day, and were considered to be developmental effects *per se* and not due to growth retardation (Heindel et al. 1992). The implication of these neurodevelopmental effects on the functional development of rats is however not clear.

**Data on barium chloride**

Test guideline prenatal developmental toxicity study in rats (Study report, 2014)

In a prenatal developmental toxicity study (OECD TG 414, GLP guidelines) female rats were administered 0, 10, 30 and 100 mg/kg bw/day throughout GD 0 – 20, via oral gavage (Study report, 2014). The rationale for the administered doses was based on a dose range finding study where mated females (n = 5/dose group) were administered 0, 50, 175 and 250 mg/kg bw/day during GD 0 – 21 via oral gavage. In the dose-range finding study, three dams from the mid-dose group and 2 dams from the high dose group died after a single administration and thus, the remaining animals were re-distributed in a mid-dose group receiving 100 mg/kg bw/day from GD 2 onwards. No effects on the implantation sites, early and late resorption or the mean number of live pups were reported in any dose group of the dose range finding study.

In the main PNDT study, two dams died on the last day of treatment at 100 mg/kg bw/day. At the same dose level, another dam showed conditional decline until GD 21, the necropsy revealing effects such as hydrothorax, haemorrhages in the liver and haemorrhagic discharge in the vagina. These maternal toxicity effects were considered treatment-related. All foetuses were found dead at necropsy in these three dams and the cause was ascribed to maternal toxicity. The mean foetus weight was comparable in all dose groups (data not shown). Foetal external, visceral and skeletal examinations did not reveal any treatment-related effects (data not shown). The NOAEL for prenatal developmental toxicity according to the findings of this study was 100 mg/kg bw/day.

Non-guideline reproductive toxicity study in rats and mice (Dietz et al. 1992)

Dietz et al. 1992 investigated the effects of barium chloride on rats administered 0, 1000, 2000 and 4000 ppm (equivalent to 0, 120, 240 and 480 mg /kg bw/day, respectively) and on mice administered 0, 500, 1000 and 2000 ppm (equivalent to 0, 90, 180 and 360 mg /kg bw/day) via drinking water. Male

rats and mice were treated for a pre-mating period of 60 days, while the females of both species were treated 30 days prior to mating. One rat dam died at the highest dose level, the necropsy revealing 7 foetuses and one resorption site. No information on maternal body weight, body weight gain or feed consumption is given for either rats or mice.

At 480 mg/kg bw/day, the rat pup weight at birth was significantly ($p<0.01$) reduced by 9%, as compared to controls. Pup survival until PND 5 was >99% for all dose levels. No external anomalies were observed in the rat offspring at any dose level.

In mice, at 180 mg/kg bw/day, a significant reduction ($p<0.05$) in litter size on PND 0 (by approx. 26%) and PND 5 (by approx. 29%) as compared to controls, was observed in the absence of maternal toxicity. Since this effect on pup viability was not seen in the high dose group, it is considered of less toxicological relevance. A few pups were found dead at birth (number not reported) for all dose groups (not specified for controls) and pup survival until PND 5 ranged between 98 – 100 % (dose level not specified, data not shown). No statistically significant changes in live pup body weights and no external abnormalities were reported.

***Conclusion on animal studies of barium chloride***

From the available studies on developmental toxicity of barium chloride in mice and rats there is not sufficient evidence to conclude that barium has the potential to exert adverse effects on development of the offspring. Thus, barium cannot be considered to be responsible for the adverse effects on the development of the offspring for barium diboron tetraoxide and the data are thus not further considered for classification purposes.

### 10.10.5.2 Human data

There is no available information on human exposure to barium diboron tetraoxide. However, data were read-across from epidemiological studies on boric acid and borate salts exposure. Several epidemiological studies performed on occupationally and/or environmentally exposed populations to boron from Turkey, Argentina, China and Hungary were assessed.

A recent retrospective cohort study was performed in order to investigate environmental boron exposure effects on women and their offspring (Duydu et al. 2018b). A total number of 199 women residing near a borate-processing plant (Bandirma) and a boron-mining plant (Bigadic Boron Works) both located in Turkey, participated in the study. Biological (i.e. blood and urine) as well as food, air and water samples were collected. Data on pregnancy outcomes were collected through a questionnaire survey that included series of questions on age, duration of marriage, preterm birth, numbers of children, birth weights of newborns, congenital anomalies, abortions, miscarriage, stillbirth, early neonatal death, neonatal death, infant death and possible confounders (smoking, alcohol consumption and pesticide application). The questionnaires were filled in by the participants and information on a total number of 326 children (162 girls and 164 boys) was collected. Based upon the measured blood boron levels, the participants were assigned into three groups: low exposure group ($n = 143$) with $39.74 \pm 27.60$ ng B/g blood and a DBE of $9.73 \pm 5.29$ mg B/day; medium exposure group ($n = 29$) with $124.19 \pm 13.10$ ng B/g blood and a DBE of $21.62 \pm 7.87$ mg B/day and high exposure group ($n = 27$) with $274.58 \pm 213.00$ ng B/g blood and a DBE of $24.67 \pm 11.39$ mg B/day. The correlation between the DBE and blood boron concentrations was statistically significant (Pearson's correlation, $p<0.01$). However, no statistically significant differences were observed when comparing the reproductive outcomes between each exposure group. No statistically significant associations between the measured blood boron levels of mothers and the birth weights of newborns were observed either. For the medium exposure group, it was found however that an increase of 1 ng B/g blood resulted in a decrease of birth weight by 4.1 g, but no statistical significance was achieved ($p>0.05$). Taking into account the results of this study, the authors concluded that environmental exposure to boron (at the above measured DBE levels) appears to be irrelevant for humans and thus, does not induce adverse effects on the development of the offspring.

As also stated by the authors, the main limitations of this study are represented by the low sample size and self-reporting of data. The time difference between the time of birth and the time of collecting the data represents another limitation, since the birth weights of the newborns were provided

retrospectively by the mothers. Therefore, the above described limitations could have impacted the statistical power of the reported results. Moreover, the highest individual blood boron concentration measured by this recent study (975.66 ng B/g blood) is evidently below the blood level (1270 ng B/g blood) corresponding to the NOAEL for developmental toxicity in rats (i.e. 9.6 mg B/kg bw/day) set by the RAC for boric acid (RAC Opinion on boric acid, 2014), based on the results of Price et al. 1997.

Furthermore, a prospective mother-child cohort study investigated environmental exposure of boron through drinking water on pregnant women residing in Salta, Argentina (Igra et al. 2016). The study was designed so that they would be seen 2-3 times, in order to repeatedly measure environmental exposure to boron via drinking water during the whole length of the pregnancies. Biological (urine and blood) and water samples as well as data on maternal age, parity (number of born children), parental monthly income, years of maternal education, smoking, alcohol consumption, chewing of coca leaves, and prenatal vitamin supplementation were gathered at the follow-up visits. The pregnancy outcomes, i.e. weight, length and head circumference, were measured at birth. Arsenic, cesium and lithium were also present in the drinking water, but were adjusted for in the statistical models used by the authors. The median serum boron concentration during pregnancy was 133 µg/L (ranging between 0.73 – 605 µg/L) and resembled the median level measured in the whole blood, i.e. 134 µg/L (ranging between 12 – 542 µg/L), while the mean urine boron level was higher, i.e. 10 494 µg/L (ranging between 1590 – 35 551 µg/L). A statistically significant ($p = 0.043$) inverse association was found between serum blood boron levels >80 µg/L and birth length, i.e. newborns were 0.7 cm shorter per each 100 µg/L increase in serum boron levels. Moreover, this association was more pronounced (increased by 28%) during the third trimester of pregnancy, when the highest serum boron concentrations were the highest (0.73 – 447 µg/L). An increase in serum boron of 100 µg/L in the third trimester was associated with a 0.9 cm reduction in length and 120 g decrease in the birth weight of newborns ($p = 0.001$ and 0.021, respectively).

As part of a more comprehensive investigation of human exposure to boron, Tuccar et al. 1998 assessed reproductive and developmental effects in families (covering three generations) living in Turkey. Three regions were identified based upon environmental boron exposure: Region I (high boron levels due to being closely located to processing plants and borate pits, with a drinking water level of 229 ppm B), Region II (located far from borate pits, with a drinking water level of 0.30 – 0.50 ppm B) and Region III (the population of this region represented a mixture of residents coming from both high- and low-exposure areas). The sample size consisted of 226 families (covering three generations) for Region I, 164 families for Region II and 177 families coming from Region III. Region II (low boron exposure) had the highest infant death rate as compared to the other regions (39 vs. 30 and 15 for Region I and Region III, respectively). Based on the gathered results (through questionnaires), the authors concluded that environmental, and both environmental and occupational exposure to boron do not induce developmental effects in humans. Nevertheless, this study presents several limitations that could have negatively influenced the results such as small sample size and the fact that prenatal development was not assessed. As presented above in Table 21, Korkmaz et al. 2007 estimated a daily exposure of 6.77 mg B/kg bw/day for the high-exposure area (Region I) which is however below the NOAEL for developmental toxicity in rats (9.6 mg B/kg bw/day).

Chang et al. 2006 investigated the developmental effects in the children born to boron mining and processing workers from China. A total number of 936 exposed workers and a comparison group composed of 251 controls participated in the study. The exposure estimates were 31.3 mg B/day and 1.4 mg B/day, which can be converted into values of 0.45 mg B/kg bw/day and 0.02 mg B/kg bw/day, respectively, assuming an average body weight of 70 kg. The evaluated parameters (i.e. stillbirths, delayed pregnancies, multiple births, spontaneous miscarriages and tubal or ectopic pregnancies) did not show any statistical difference from controls. Thus, the authors concluded that the offspring coming from parents occupationally exposed to boron do not present developmental toxic effects. However, since no exposure measurements were carried out for the wives of the workers, this conclusion is solely based on the occupational exposure of the fathers, which is below the NOAEL for developmental toxicity in rats (9.6 mg B/kg bw/day).

Acs et al. 2006 carried out a case-control study in Hungary in order to investigate the occurrence of congenital abnormalities of infants born to mothers using vaginal tablets containing boric acid as treatment for infectious diseases of the genital organs (two tablets of 30 mg each, daily for 7 days). A percentage of 0.14 mothers (52 out of 38 151) from the control group and 0.19% (43 out of 22 843) from the study group received boric acid tablets. The results of this study show a higher risk of neural tube defect (2 cases) when the exposure to boric acid appeared during the second and third months of pregnancy. In addition, skeletal defects occurred in the infants of mothers exposed to boric acid treatment for the whole length of the pregnancy. The authors conclude that boric acid might have a weak teratogenic potential, but taking into account the reduced absorption through topical exposure, this would most likely appear in the case of a damaged vaginal epithelium.

***Conclusion on the human studies of boron***

The only prospective mother-child cohort study available on boron environmental exposure shows a significant inverse association between serum boron levels >80 µg/L and birth size. Moreover, this association increased by 28% in the third trimester of pregnancy, when the serum boron concentrations were higher. The serum boron concentrations during pregnancy ranged between 0.73 – 605 µg/L (median of 133 µg/L) and correlated strongly with the whole-blood boron levels (Igra et al. 2016). A serum level of 80 µg/L would correspond to 75 ng B/g blood (blood density level of 1060 kg/m$^3$), which is lower than the blood level (1270 ng B/g blood) associated with the NOAEL of developmental toxicity in rats (Price et al. 1997). These recent epidemiological data thus indicate that environmental exposure to boron might have an adverse effect on the development of offspring. However, it cannot be excluded that the observed effects can be the result of a combined exposure to lithium.

The other retrospective studies on environmental and/or occupational boron exposure did not provide clear evidence of developmental effects. These studies are however associated with limitations such as small sample size, self-reporting of data, and the fact that high boron exposure levels were still lower than the NOAEL for developmental toxicity in rats. The epidemiological data do not contradict the animal data and therefore, there is no reason to question the relevance for humans of the developmental toxicity observed in the animal studies.

### 10.10.6 Comparison with the CLP criteria

As stated in the CLP Regulation (EC) No 1272/2008, the classification of substances in category 1A for reproductive toxicity (known human reproductive toxicant) is "*largely based on evidence from humans*". No human data on developmental toxicity of barium diboron tetraoxide are available, but read-across epidemiological information on developmental toxicity of boron was assessed. These studies do not provide clear evidence of developmental effects and present various methodological limitations that might have influenced the reported results. Therefore, classification of barium diboron tetraoxide as Repr. 1A is not appropriate.

In accordance with the CLP criteria for classification, the available animal data provide clear evidence of developmental toxicity manifestations (death of the developing organism, structural abnormality and altered growth) in different species (i.e. rats, mice and rabbits). These are primarily expressed as severely decreased foetal weight observed in the absence of maternal toxicity, effects on the CNS that are not due to growth retardation, anomalies of the eyes, and cardiovascular and skeletal malformations. While the most common developmental effects (i.e. agenesis or shortening of rib XIII) were observed in both rats and mice, the developmental cardiovascular effects (i.e. interventricular septal defects, enlarged aorta, pulmonary artery and aorta arising from the right ventricle) were seen only in rabbits.

Furthermore, an increased prenatal mortality consistent across species was observed, i.e. 73% for rabbits, 36% for rats and 19% for mice at 44, 94 and 111.3 mg B/kg bw/day, respectively. The available data indicate that the rat is the most sensitive species to boron developmental effects, with an overall NOAEL of 9.6 mg B/kg bw/day.

Therefore, based on the observed developmental toxic effects that are not considered to be non-specific secondary consequences of maternal toxicity, classification of barium diboron tetraoxide as **Repr. 1B, H360D** is warranted.

***Concentration limits***

In line with the CLP guidance (2017), concentration limits for developmental toxicity are derived by calculating the reproductive toxicity dose descriptor, i.e. ED10 (the dose level at which a change of 10% compared to the concurrent control group is observed). It should be noted that the available data on barium diboron tetraoxide itself were not sufficient in order to derive the ED10 since there was no clear evidence of developmental toxicity, and thus read-across data on boric acid and borate salts were used.

According to the RAC (RAC opinions on boric acid, disodium octaborate anhydrate and disodium octaborate tetrahydrate, 2014), increased incidence of short rib XIII considered as a malformation, was identified as the most sensitive effect on the development of the offspring in rats, based upon a prenatal developmental toxicity study with boric acid (Price et al. 1996a). As also stated by RAC in the respective opinions on boric acid and borate salts in 2014, the foetal incidence of short rib XIII was 1.2% at 13.3 mg B/kg bw/day (LOAEL) and 1.5% at 25 mg B/kg bw/day. Since the incidences are low, an ED10 cannot be derived and thus, the LOAEL of 13.3 mg B/kg bw/day is used for setting the concentration limits.

Correcting for the percentage of boron, a level of 13.3 mg B/kg bw/day would correspond to 147.8 mg barium diboron tetraoxide/kg bw/day. Barium diboron tetraoxide is therefore assigned to the medium potency group with a GCL of 0.3% since the ED10 is ≥ 4 mg/kg bw/day and ≤ 400 mg/kg bw/day.

***Conclusion***

Setting of specific concentration limit for adverse effects on the development of the offspring is not considered justified, and thus the GCL of 0.3% applies.

### 10.10.7 Adverse effects on or via lactation

No information was available for the assessment of effects on or via lactation of barium diboron tetraoxide. Read-across data on boric acid and borate salts from multi-generation animal studies are presented below. The available epidemiological studies investigating boron environmental and/or occupational exposure did not provide information on this endpoint. No animal data on barium effects on or via lactation were available.

**Table 23: Summary table of animal studies on effects on or via lactation**

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group[23]</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td colspan="4">Boric acid and borax (disodium tetraborate decahydrate)</td></tr>
<tr><td>Reproductive toxicity assessment study<br><br>No guideline specified, but conforms to the standard three-generation, 2</td><td>Test material: boric acid or borax<br><br>Purity: unknown<br><br>Doses/conc.: 0, 117, 350 and 1170 ppm boron,</td><td>Effects on or via lactation<br>Significantly higher (p<0.05) lactation indices were observed at 5.9 and 17.5 mg B/kg bw/day, for both boric acid and borax treatments, and at 17.5 mg B/kg bw/day, the P3-F3A generation administered borax showed a significantly (p<0.05) lower lactation index than controls (presented below).<br><br><table><tr><th>Index</th><th>Control</th><th>5.9 mg B/kg</th><th>17.5 mg B/kg bw/day</th><th>Control</th><th>5.9 mg B/kg bw/day</th><th>17.5 mg B/kg</th></tr></table></td><td>Weir and Fisher 1972<br><br>Weir 1966</td></tr>
</table>

[23] Where applicable and unless stated otherwise, the reliability scores of the studies presented in Table 23 are according to the CLH dossier of boric acid, assessed by RAC in 2013.

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group[23]</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>litters per generation multi-generation studies normally used at the time.<br><br>The first filial generation (F1A) was carried through weaning and discarded. The parental generation (P1) was rebred to produce their second litter (F1B). At the time of weaning, 16 females and 8 males each from the control and test groups were selected at random and designated the second parental generation (P2) for continuation of the reproduction study. These animals were bred to produce the F2A and F2B litters as before. The F2B litter became the P3 generation and were bred to produce the F3A and F3B litters.<br><br>Rat (Sprague-Dawley) male/female</td>
<td>equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw<br><br><u>Exposure</u>: from the beginning of the study (14 weeks pre-mating exposure) until sacrifice of parents P1, and from weaning until sacrifice of the F1- and F2-generations (daily in feed).</td>
<td>
<table>
<tr><th></th><th></th><th>bw/day</th><th></th><th></th><th></th><th>bw/day</th></tr>
<tr><td colspan="7">Borax</td></tr>
<tr><td rowspan="11">Lactation index[a]</td><td colspan="3">P1-F1A</td><td colspan="3">P1-F1B</td></tr>
<tr><td>56.3</td><td>63.6</td><td>82.3[b]</td><td>58.8</td><td>60</td><td>74.2</td></tr>
<tr><td colspan="3">P2-F2A</td><td colspan="3">P2-F2B</td></tr>
<tr><td>48.3</td><td>79.8b</td><td>82.7[b]</td><td>92.1</td><td>93.2</td><td>95.5</td></tr>
<tr><td colspan="3">P3-F3A</td><td colspan="3">P3-F3B</td></tr>
<tr><td>91.5</td><td>81.1</td><td>79.1[c]</td><td>89.7</td><td>91.8</td><td>95.9</td></tr>
<tr><td colspan="7">Boric acid</td></tr>
<tr><td colspan="3">P1-F1A</td><td colspan="3">P1-F1B</td></tr>
<tr><td>56.3</td><td>96.2</td><td>70.3[b]</td><td>58.8</td><td>85.6b</td><td>80[b]</td></tr>
<tr><td colspan="3">P2-F2A</td><td colspan="3">P2-F2B</td></tr>
<tr><td>48.3</td><td>79.2[b]</td><td>83.1[b]</td><td>92.1</td><td>81</td><td>98</td></tr>
<tr><td colspan="3">P3-F3A</td><td colspan="3">P3-F3B</td></tr>
<tr><td>91.5</td><td>82.5</td><td>86.5</td><td>89.7</td><td>86.7</td><td>87.9</td></tr>
</table>
[a] Lactation index = number of weaned pups/number left to nurse x 100.<br>
[b] Significantly higher than controls.<br>
[c] Significantly lower than controls.
</td>
<td></td>
</tr>
</table>

| **Method, guideline, deviations if any, species, strain, sex, no/group[23]** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| n = 8 males/dose group and 16 females/dose group<br><br>Reliability: 2 (reliable with restrictions) | | | |
| **Reproductive assessment by continuous breeding**<br><br>Performed according to the NTP's Reproductive Assessment by Continuous Breeding Protocol<br><br>Mouse (Swiss) male/female<br><br>n = 19/sex/dose groups<br><br>No litters were born to F0 parents exposed to 9000 ppm, and only three litters were born alive to the 4500 ppm breeding pairs after cohabitation ended. Thus, F1 animals in the control and 1000 ppm groups were chosen for assessing the F1 generation.<br><br>Reliability: 2 (reliable with | Test material: boric acid<br><br>Purity: >99%<br><br>Doses/conc.: 0, 1000 ppm, 4500 ppm or 9000 ppm equivalent to 0, 152, 636 and 1262 mg boric acid/kg bw/day, equivalent to 0, 26.6, 111.3 and 221 mg B/kg bw/day, respectively.<br><br>Exposure: 27 weeks (daily in feed) | **Effects on or via lactation**<br>During the lactation period, there were no effects on viability or growth of F1 or F2 pups at any dose level. | Fail et al. 1991 |

| **Method, guideline, deviations if any, species, strain, sex, no/group[23]** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| restrictions) | | | |
| **Prenatal Developmental Toxicity Study**<br><br>GLP-compliant<br><br>Rat (Crl: CD VAF/Plus (Sprague Dawley))<br><br>n = groups of 14 – 17 females/dose group/phase<br><br>Reliability: 1 (reliable without restriction), key study<br><br>In phase II the dams were allowed to deliver and the pups reared to weaning and then killed for full visceral and skeletal examination. | Test material: boric acid<br><br>Purity: 98%<br><br><u>Doses/conc.:</u> 0, 250, 500, 750, 1000, 2000 ppm boric acid equivalent to 0, 19, 36, 55, 76 and 143 mg boric acid/kg bw/day, respectively (equivalent to 0, 3.3, 6.3, 9.6, 13.3 and 25 mg B/kg bw/day)<br><br><u>Exposure phase II</u>: days 0 - 20 post mating (nominal in diet), then on normal diet until termination on PND 21 | **Effects on or via lactation**<br>During lactation and until PND 21, there were no effects on viability or growth of the offspring at any dose level. | Price et al. 1996a |

## Table 24: Summary table of human data on effects on or via lactation

| **Type of data/report** | **Test substance,** | **Relevant information about the study (as applicable)** | **Observations** | **Reference** |
|---|---|---|---|---|
| No human studies showing effects on or via lactation were available. | | | | |

## Table 25: Summary table of other studies relevant for effects on or via lactation

| **Type of study/data** | **Test substance,** | **Relevant information about the study (as applicable)** | **Observations** | **Reference** |
|---|---|---|---|---|
| No other studies relevant for effects on or via lactation were available. | | | | |

### 10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

#### 10.10.8.1 Animal studies

**Data on barium diboron tetraoxide**

No information on the effects of barium diboron tetraoxide on or via lactation was available.

**Data on boric acid and borate salts**

In a three-generation study (Weir and Fisher 1972) performed in rats administered boric acid or borax via feed, significantly ($p<0.05$) higher lactation indices (i.e. higher rate of surviving pups from birth to weaning) were observed for F1 and F2 generations (by approx. 34% and 71%, respectively, as compared to controls), at 5.9 and 17.5 mg B/kg bw/day. However, at 17.5 mg B/kg bw/day administered as borax in the F3 generation, a significantly ($p<0.05$) decreased lactation index was observed (by approx. 14%, as compared to controls). This effect was not seen at an equivalent dose of boric acid. The filial generations (F1, F2 and F3) did not differ statistically significantly from controls in terms of litter size, foetal weight and external appearance during lactation (data not shown). No information on maternal toxicity was reported. Due to the ambiguous data on pup viability during the lactation periods, and the unusually low survival rate in control pups of F1 and F2 generations, these data are not considered sufficient for classification for effects via lactation.

In a multi-generation study in mice administered boric acid (NTP continuous breeding protocol), no statistically significantly differences were observed in the body weight or viability of the F1 or F2 pups in any dose group, as compared to control pups, during lactation.

**Data on barium chloride**

No information on the effects of barium chloride on or via lactation was available.

#### 10.10.8.2 Human data

**Data on boric acid and borate salts**

Administered doses of 1 – 13 g boric acid to breastfeeding mothers, gave rise to levels of 10 – 250 mg/L B in breast milk (Moseman 1994).

**Data on barium compounds**

No information on the effects of barium on or via lactation was available.

### 10.10.9 Comparison with the CLP criteria

As stated in the CLP Regulation (EC) No 1272/2008, the classification of substances for effects on or via lactation is assigned on the *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 effects in the offspring due to transfer in the milk or adverse effects 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 was no human evidence indicating a hazard of barium diboron tetraoxide or boron to babies during the lactation period.

There was no evidence of adverse effects in the offspring due to transfer in the milk or adverse effects on the quality of the milk in the available multi-generational studies of boric acid and borax in mouse and rat.

Based on the Moseman (1994) study, the data are not sufficient to conclude that boron is present in potentially toxic levels in breast milk.

Therefore, classification of barium diboron tetraoxide for adverse effects on or via lactation is not warranted.

### 10.10.10 Conclusion on classification and labelling for reproductive toxicity

Based on the total weight of evidence, classification as toxic to reproduction in category 1B for adverse effects on sexual function and fertility and the development of offspring, with a GCL of 0.3% for both hazard classes is considered appropriate.

Thus, the resulting classification proposal for barium diboron tetraoxide is **Repr. 1B, H360FD.**

## 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***

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

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<br>Boric acid, borax<br>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)<br>Females: reduced ovary weight (absolute by 27%/42%, relative by 16%/32% b.a./borax) | Clinical signs, reduced bw (males by 44%/55%, females by 12/10% b.a./borax) |
| | 47 | 490 / 450 | Males: partial testes atrophy (5 animals out of 20?) | None |
| 90-day, dog, dietary<br>Boric acid, borax<br>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<br>Boric acid, borax<br>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.<br>Males: testes atrophy and lack of viable sperm (all animals)<br>Females: decreased ovulation in approx. half of the examined ovaries | Reduced bw (data not shown) |
| 9-week, investigation of testicular toxicity, rat, dietary<br>Boric acid<br>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%), | Reduced bw by 16% |

| | | | | |
|---|---|---|---|---|
| | | | epididymis weight (by up to 57%) and epididymal sperm count (by up to 97%); increased FSH and LH | |
| | 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<br>Boric acid<br>Treinen and Chapin, 1991 | 189 | 1900 / 1800 | Inhibited spermiation, peripheral spermatid nuclei, epithelial disorganisation, cell exfoliation, luminal occlusion, cell death, significant loss of spermatocytes and spermatids from all stage tubules (all animals); decreased basal testosterone level (by 69%) | Reduced bw by 8% |
| Continuous breeding, mouse, dietary<br>Boric acid<br>Fail *et al.*, 1991 | 221 | 2300 / 2100 | None of the F0 pairs was fertile<br>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<br>Males: tubular degeneration, reduced testis weight (by 51%), reduced epididymis and prostate weight, reduced epididymal sperm count (by 72%), decreased percentage of motile | Reduced bw (females by 7%) |

| | | | | |
|---|---|---|---|---|
| | | | sperm, increased percentage of abnormal sperm (ca. 5-fold) | |
| 60-day, investigation of male fertility, rat, dietary<br>Borax<br>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)<br>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<br>Heindel *et al.*, 1992<br>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 limb; wavy rib, reduced ossification | None |
| | 29 | 300 / 280 | Reduced foetal weight (by 13%); increased incidence of short rib XIII; wavy rib | None |
| PNDT, rat, dietary<br>Price *et al.*, 1996a<br>(follow-up study to Heindel *et al.*, 1992)<br>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<br>Phase II: short rib XIII | None |
| PNDT, mouse, dietary | 175 | 1800 / 1700 | Increased resorptions (19% vs 6%), reduced foetal weight (by 33%); increased incidence of | Renal tubular dilation; corrected |

| | | | | |
|---|---|---|---|---|
| Heindel *et al.*, 1992<br>Dosing GD 0-17 | | | short rib XIII | bw not affected |
| PNDT, rabbit, gavage<br>Price *et al.*, 1996b<br>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<br>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 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.

<u>Conclusion on classification</u>

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**.

### 10.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier.

### 10.12 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier.

### 10.13 Aspiration hazard

Hazard class not assessed in this dossier.

## 11 EVALUATION OF ENVIRONMENTAL HAZARDS

Enivironmental hazards were not assessed in this dossier.

## 12 EVALUATION OF ADDITIONAL HAZARDS

Additional hazards were not assessed in this dossier.

## 13 ADDITIONAL LABELLING

Not relevant.

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BARIUM DIBORON TETRAOXIDE

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## 15 ANNEXES

Annex I to the CLH Report

CONFIDENTIAL Annex: Constituents and concentration range