CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name: Magnesium metaborate

EC Number: 237-235-5

CAS Number: 13703-82-7

Index Number: -

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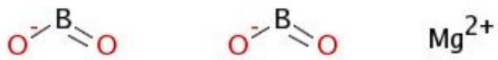
CONTENTS

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)	Magnesium metaborate
Other names (usual name, trade name, abbreviation)	Boric acid (HBO2), magnesium salt (2:1)
ISO common name (if available and appropriate)	Not applicable
EC number (if available and appropriate)	237-235-5
EC name (if available and appropriate)	Magnesium metaborate
CAS number (if available)	13703-82-7
Other identity code (if available)	Not available
Molecular formula	B2MgO4
Structural formula	
SMILES notation (if available)	[Mg+2].O=B[O-].O=B[O-]
Molecular weight or molecular weight range	109.925
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 applicable

As specified in **Annex VI of CLP 1.1.1.3.**:

It should be noted that the EINECS number includes both anhydrous and hydrated forms of a substance, and there are frequently different CAS numbers for anhydrous and hydrated forms. The CAS number included is for the anhydrous form only, and therefore the CAS number shown does not always describe the entry as accurately as the EINECS number.

As a consequence, the proposed entry for **EC 237-235-5** in Annex VI covers both the **anhydrous and all hydrated forms** 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 (CLP)	Current self-classification and labelling (CLP)
Magnesium metaborate EC number: 237-235-5 CAS number: 13703-82-7	CONFIDENTIAL	Not included in Annex VI	Skin Sens. 1B, H317, SCL (%): > 15 - < 100

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 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
CONFIDENTIAL				No

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
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2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	**Hazard statement Code(s)**	**Pictogram, Signal Word Code(s)**	**Hazard statement Code(s)**	**Suppl. Hazard statement Code(s)**		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	TBD	magnesium metaborate	237-235-5	13703-82-7	Repr. 1B	H360FD	GHS08 Dgr	H360FD			Note 11[#]

Current draft for Note 11. To be confirmed by the Commission Regulation. Note 11: The classification of mixtures as reproductive toxicant is necessary if the sum of the concentrations of individual boron compounds that are classified as reproductive toxicant in the mixture as placed on the market is ≥ 0.3 %.

Table 6: Reason for not proposing harmonised classification and status under consultation

Hazard class	Reason for no classification	Within the scope of 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	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
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	Harmonized 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

Magnesium metaborate has not previously been discussed and/or agreed by the TC C&L (Dir. 67/548/EEC) and is not included in CLP Annex VI. Magnesium metaborate was included in a Group Regulatory Strategy of inorganic borates during the year of 2020 by the SE CA (current dossier submitter) where a concern for reproductive toxicity was confirmed for 10 registered and one associated unregistered, currently unregulated members of the inorganic borates group. This group of inorganic borates are expected to generate boric acid upon hydrolysis. Boric acid has a harmonized classification as Repr.1B, H360FD. Within the borate group, substances based on alkali metals, alkaline earth metals or ammonium counter ions were included. These substances are expected to show moderate water solubility and the associated counter ions have no or low toxicity. The majority of registrants of these substances use read-across from boric acid and borate salts to fill data gaps for effects on fertility and development. The SE CA considers the read-across approach from boric acid and borate salts to be valid and sufficient for harmonized classification in Repr. 1B, H360FD for all 11 identified inorganic borates. For magnesium metaborate, however, read-across for reproductive toxicity is not applied by the registrant(s) since the substance has substance specific data from an OECD TG 422 screening study of reproductive toxicity and the registrant(s) has not self-classified the substance for reproductive toxicity. In contrast, the SE CA considers that the data of magnesium metaborate does not contradict the data of boric acid and borate salts and thus classification in category 1B is warranted based on a Weight of Evidence approach using substance specific data and read-across from boric acid and borate salts. Hence, an appropriate regulatory action for the 11 inorganic borates is harmonized classification and labelling with subsequent inclusion in Annex VI to CLP. Accordingly, 11 separate CLH-proposals will be submitted simultaneously.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level. Magnesium metaborate is considered to fulfil the criteria for classification as toxic to reproduction (Repr. 1B, H360FD). Therefore, a harmonised classification is justified according to Article 36(1)(d) of the CLP Regulation.

The proposed classification and labelling of magnesium metaborate for reproductive toxicity is based on a weight of evidence determination of substance specific data from an OECD TG 422 screening study of reproductive toxicity and 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

Magnesium metaborate is mainly used in lubricants and greases in vehicles or machinery.

6 DATA SOURCES

Information on magnesium metaborate and read-across data included in the present CLH-report originates from the publicly disseminated REACH Registration Dossier (ECHA, 2021) and RAC Opinions on boric acid, disodium tetraborate anhydrate and disodium octaborate tetrahydrate (ECHA, 2014a;b;c), as well as RAC opinions on barium diboron tetraoxide (ECHA, 2020) and on the revision of concentration limits for reproductive toxicity of boric acid and a number of borates (ECHA, 2019). Additional relevant studies included in CLH-proposals of sodium per(oxo)borates (ECHA, 2021a,b,c) and of trimethyl borate (ECHA, 2021d) and relevant studies available in the scientific literature have also been included.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20°C and 101,3 kPa	liquid	REACH registration (ECHA dissemination, 2021)	
Melting/freezing point	-12° C	REACH registration (ECHA dissemination, 2021)	Measured
Boiling point	162° C	REACH registration (ECHA dissemination, 2021)	Measured
Relative density	-	REACH registration (ECHA dissemination, 2021)	
Vapour pressure	0,036 Pa at 25° C 0,091 Pa at 40° C 0,608 Pa at 70° C	REACH registration (ECHA dissemination, 2021)	Measured
Surface tension	<= 67 mN/m at 21 °C and a loading rate of 5.5 g/L	REACH registration (ECHA dissemination, 2021)	Measured
Water solubility	magnesium: < 1 x 10E-4 g Mg/L boron: < 1 x 10E-4 g B/L At 20.0 °C and a nominal loading rate of 0.1 g/L	REACH registration (ECHA dissemination, 2021)	Measured
Partition coefficient n-octanol/water	-	REACH registration (ECHA dissemination, 2021)	
Flash point	164 °C at 101 325 Pa	REACH registration (ECHA dissemination, 2021)	Measured
Flammability	-	REACH registration (ECHA dissemination, 2021)	
Explosive properties	Not explosive	REACH registration (ECHA dissemination, 2021)	Based on the chemical structure of the test item the result for the explosive properties has been predicted negative.
Self-ignition temperature	416 °C at 101 325 Pa	REACH registration (ECHA dissemination, 2021)	Measured
Oxidising properties	Not oxidising	REACH registration (ECHA dissemination, 2021)	Based on the chemical structure of the test item the result for the oxidizing properties has been predicted negative.
Granulometry	-	REACH registration (ECHA dissemination, 2021)	

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Stability in organic solvents and identity of relevant degradation products	-	REACH registration (ECHA dissemination, 2021)	
Dissociation constant	-	REACH registration (ECHA dissemination, 2021)	
Viscosity	-	REACH registration (ECHA dissemination, 2021)	

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this CLH proposal.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	**Results**	**Remarks**[1]	**Reference**
	Human data		
Boric acid and borate salts			
***In vivo* percutaneous absorption study in humans** 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.	*In vivo* dermal absorption: 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. 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. 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 Purity: unknown Reliability: 1	Wester et al. 1998a
Percutaneous absorption through human skin *in vitro* *In vitro* diffusion from aqueous solution was determined in receptor fluid accumulation over a 24h period. Human cadaver skin	Dermal absorption: 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	Test material: boric acid, disodium tetraborate decahydrate, disodium octaborate tetrahydrate Purity: unknown	Wester et al. 1998b

[1] Where applicable and unless stated otherwise, the reliability scores of the studies presented in Table 8 are according to the CLH dossier of boric acid, assessed by RAC in 2014.

Method	Results	Remarks[1]	Reference
(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. 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. Changes in boron isotope ratios by IPCMS (Inductively Coupled Plasma-Mass Spectrometry) were used to measure absorption.	volume), flux decreased some 200-fold to 0.07 $mg/cm^2/hr$ and Kp of 1.4 x 10^{-6} cm/hr. Borax (disodium tetraborate decahydrate) dosed at 5 %/1000 $\mu L/cm^2$ had 0.41 % dose absorbed. Skin surface wash recovery was 87.7 ± 5.9 % dose. Flux was 8.5 $\mu g/cm^2/h$, and Kp was 1.7 x 10^{-4} cm/h. Disodium octaborate tetrahydrate dosed at 10 % /1000 $\mu L/cm^2$ was 0.19 % dose absorbed. Skin surface wash recovery was 91.3 ± 25.2 % dose. Flux was 0.8 x 10^{-4} cm/h. 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).	Reliability: 1	
Dermal absorption in infants 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 Purity: unknown Reliability: 2	Friis-Hansen et al. 1982
Boron			
Literature review of published and proprietary data	<u>Absorption</u>: inhaled boron is absorbed and systemically distributed, almost complete gastrointestinal absorption following oral exposure. <u>Distribution:</u> widely distributed throughout the body including reproductive tissues but has a low affinity for fat. At high doses, boron accumulates in the bone. <u>Metabolism</u>: being an inorganic element, boron is not metabolised by humans, but the parent borate is recovered in the blood, tissues and urine. <u>Elimination and excretion</u>: 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	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

Method	Results	Remarks[1]	Reference
	(range 4 – 27.8 h) in nine cases of boric acid poisoning.		
***In vivo* human excretion of boron, specifically examining renal clearance** 16 pregnant women in the 2nd trimester (14 – 28 weeks) and 15 nonpregnant women (designated as age-matched references). Blood samples for boron, creatinine and urea were collected at the start, at 2 h and 24h. Urine was collected during the first 2h in the Clinical Research Centre and during 22 h outside the centre for measurement of volume, boron and creatinine.	The pregnant and non-pregnant boron intake was 1.35 mg boron/24h and 1.31 mg boron/24h, respectively. Renal clearance for 2h period: 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. Renal clearance for 24h period 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. Plasma levels: 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. Differences in the serum creatinine clearances indicated that urine collection had not been complete over the entire 24 h collection period. Comparison of renal boron clearance with creatinine clearance indicated that tubular reabsorption of boron occurred in both pregnant and non-pregnant women.	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). Purity: unknown Reliability: 1	Pahl et al. 2001
Neutron activation analysis-electrothermal atomic absorption spectroscopy (ETA-AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) analysis 46 elements from urine, blood and serum of unexposed Italian subjects	Boron was not present in the blood or serum of healthy Italian subjects. 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. The reference values were 9490 - 3290 µg/L and range of uncertainty was > 3290 – 7800 µg/L.	Environmental exposure to boron Reliability: 2	Minoia et al. 1990

Method	Results	Remarks[1]	Reference
living in the same region, were determined. The subjects were considered representative of five subgroups resident in urban, suburban, rural and low and high hill areas. 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 the municipal supply and mineral water used.	The upper limit for metabolic anomalies was > 7800 µg/L.		
Animal data			
Boric acid			
Rat (Sprague - Dawley), female n $_{\text{(renal clearance study)}}$ = 10 non-pregnant/group and 10 pregnant/group n $_{\text{(half-life study)}}$ = 6 non-pregnant/group and 6 pregnant/group Exposure: oral (gavage), single administration Doses/conc.: - 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. -Plasma half-life study: 30 mg boric acid/kg, equivalent to 5.24 mg B/kg bw.	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). The 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). Half-life: the plasma half-life of boric acid in non-pregnant and pregnant rats given boric acid by gavage was 2.93 ± 0.24 and 3.23 ± 0.28 hours, respectively. Identified metabolites: none, boric acid is not metabolised. The authors concluded that pregnancy did not induce a statistically significant alteration of the renal clearance or plasma half-life of boron in rats.	Test material: boric acid Purity: > 99% Reliability[2]: 1	Vaziri et al. 2001 REACH registration (ECHA dissemination, [2018])
Rat (Fischer 344) male oral: feed n = 6/dose group Exposure: oral (feed), for 9 weeks Doses/conc.: 0, 3000, 4500,	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. 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.	Test material: boric acid Purity: 99.99% Reliability: 2	Ku et al. 1993

[2] The reliability score for this study is according to the publicly 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[1]	Reference
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.	Identified metabolites: none, boric acid is not metabolised.		
Rat (Fischer 344), male n = 30/group Exposure: oral (feed), daily for 7 days 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, except for bone and adipose tissue. Bone boron levels continued to increase up to the termination at 7 days (40 – 50 µg/g by day 7). **Boron levels in examined tissues** Control boron levels in plasma and all tissues examined were below 4 µg/g (range 0.66-3.69 µg/g), except for adrenal glands (7.99 µg/g): - Plasma 1.94 ± 0.17; - Liver 0.66 ± 0.10; - Kidney 1.55 ± 0.03; - Adipose tissue 1.71 ± 0.17; - Muscle 3.69 ± 0.54; - Bone 1.17 ± 0.19; - Large intestine 3.08 ± 0.17; - Brain 0.76 ± 0.02; - Hypothalamus 0.91; - Testis 0.97 ± 0.10; - Epididymis 0.81 ± 0.15; - Seminal vesicles 1.64 ± 0.23; - Seminal vesicle fluid 2.05; - Adrenals 7.99; - Prostate 1.20. Day 1 (µg B/g tissue, compared to controls): - bone showed a 20-fold increase (i.e., 23.57 ± 1.19); - 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); - 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); - 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). All 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	Test material: boric acid Purity: unknown Reliability: 2	Ku et al. 1991

Method	Results	Remarks[1]	Reference
	± 0.05 (mean ± SE) at both days 4 and 7, excluding bone and adipose tissue. <u>Days 4 - 7 (compared to controls):</u> - bone showed a 37-fold increase (i.e., 16.37 ± 1.42 - 16.00 ± 0.71); - 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); - 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); - 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); - adipose tissue showed a 2-fold increase, i.e. 3.45 ± 0.22 - 3.78 + 0.13. <u>Identified metabolites</u>: none, boric acid is not metabolised.		
Literature review of published and proprietary data	<u>Absorption</u>: oral absorption fraction in rats was found at 95%. Boron is readily absorbed through damaged skin in rabbits. <u>Distribution</u>: in male rats, boron is evenly distributed to liver, kidney, brain, muscle, adrenals, epididymis, testes, seminal vesicles, and blood, but not fat, following 61 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. <u>Metabolism</u>: being an inorganic element, boron is not metabolised by animals, but the parent borate is recovered in the blood, tissues and urine. <u>Elimination and excretion</u>: excretion primarily through renal elimination, with a renal clearance value of 163 mg/kg/ hour in rats.	The report considered experimental animal 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), which occurred through various routes of exposure (i.e., inhalation, oral, dermal, intravenous and intra-tympanic).	ATSDR report, (2010)
Comparative review of the toxicokinetics of boric acid in humans and animals			
Literature review of published data	<u>Absorption</u>: - Oral absorption: humans and animals (rats, rabbits, sheep and cattle) absorb boric acid similarly, i.e., readily and completely from the gastrointestinal tract. - Dermal absorption: negligible absorption	The review considered both human and experimental animal exposure to boric acid,	Murray 1998

Method	Results	Remarks[1]	Reference
	across intact skin for both animals and humans; for non-intact skin, the absorption varies with the used vehicle. 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: - 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. - Similar to humans, the highest level 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. 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. 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. 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).	which occurred through various routes of exposure (i.e., oral, dermal, intravenous).	

9.1 Justification for read-across from boric acid and borate salts

There is no available *in vivo* information on the toxicokinetic properties of magnesium metaborate and there is only a OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of magnesium metaborate available for the hazard class (reproductive toxicity) assessed in this CLH proposal. Classification for reproductive toxicity following oral exposure is based on a weight of evidence determination using substance specific data and 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.

Magnesium metaborate is the inorganic ionic salt of boric acid. Magnesium metaborate is described to have low solubility in water. The solubility of magnesium metaborate in water is $< 1 \times 10^{-4}$ g/L at 20 °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, Mg^{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, magnesium metaborate 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 (Mg^{2+}). The relative concentrations of the boron species are 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)[3], studies performed with rats, rabbits, sheep and cattle 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/litre 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.

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.

9.2 Toxicokinetic data on boric acid and borate salts

No studies according to validated test guidelines on the toxicokinetics of magnesium metaborate, boric acid or borate salts are available. The data described above in Table 8 are mainly represented by what is available in the open scientific literature as experimental (animal data) and occupational studies, and literature reviews.

Absorption

Oral

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 absorbed across pulmonary tissues and into the bloodstream.

3 http://www.inchem.org/documents/ehc/ehc/ehc204.htm#PartNumber:6

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 in humans. In rats, adipose tissue levels of boron represented only 20% of the plasma levels whereas testis levels of boron in male rats were almost equal to the levels 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.

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

When exposed via the oral or inhalational route borates are easily absorbed (up to 100%) into the blood stream and distributed throughout the tissues and organs of the body. By dermal exposure, an uptake of 0.5% over intact skin is considered as a maximum uptake. Boric acid is not metabolized in the body but is excreted as such mainly via the urine, with an elimination half-life of less than 24 hours in humans.

In aqueous solutions at physiological and acidic pH, low concentrations of simple borates such as magnesium metaborate will predominantly exist as undissociated boric acid. Above pH 10 the metaborate anion $B(OH)_4$ becomes the main species in solution. The toxicokinetics and toxicological effects of systemic boric acid and magnesium metaborate will therefore be expected to be similar on a boron equivalents basis.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity – oral route

Not evaluated in this CLH proposal.

10.2 Acute toxicity - dermal route

Not evaluated in this CLH proposal.

10.3 Acute toxicity - inhalation route

Not evaluated in this CLH proposal.

10.4 Skin corrosion/irritation

Not evaluated in this CLH proposal.

10.5 Serious eye damage/eye irritation

Not evaluated in this CLH proposal.

10.6 Respiratory sensitisation

Not evaluated in this CLH proposal.

10.7 Skin sensitisation

Not evaluated in this CLH proposal.

10.8 Germ cell mutagenicity

Not evaluated in this CLH proposal.

10.9 Carcinogenicity

Not evaluated in this CLH proposal.

10.10 Reproductive toxicity

There is one combined repeated dose toxicity study with reproduction/developmental toxicity screening (OECD TG 422) of magnesium metaborate available for reproductive toxicity in the registration dossier and no read-across from boric acid and borate salts is applied by the registrant(s). However, according to the dossier submitter, there is no scientific evidence to conclude that read-across from boric acid and borate salts to magnesium metaborate, similar to other inorganic borate salts, would not be justified for systemic endpoints. Therefore, for the purpose of classification, read-across from boric acid and borate salts is used together with the substance-specific information in a WoE determination.

With the exception of a recent study investigating the effects of boric acid on rat fertility (Marat et al. 2018), and a sub-acute study of the effects of boric acid on testes in mouse (Aktas et al. 2020) the studies of boric acid and borax given in Table 9 below were appointed key studies by the RAC in its 2014 opinions on boric acid, disodium octaborate anhydrate and disodium octaborate tetrahydrate, all conclusive (by consensus) on Repr. 1B (H360 FD) classifications (ECHA, 2014a, b, c) . The study by Marat et al. (2018) was included in the CLH-proposal for barium diboron tetraoxide (ECHA, 2020) and the study by Aktas et al. (2020) was included in the CLH-proposal of trimetyl borate (ECHA, 2021d). Several human studies on the effects of boron on male fertility has been published since the adoption of the RAC opinions in March 2014 and some of these were included and discussed in the CLH-proposal for revising concentration limits for reproductive toxicity of boric acid and a number of borates (ECHA, 2019) as well as in the CLH proposal of barium diboron tetraoxide (ECHA, 2020). Additional studies not included in previous CLH-proposals and RAC-opinions were included in the CLH-proposals of sodium per(oxo)borates (ECHA, 2021a,b,c) and of trimethyl borate (ECHA, 2021d) and were adapted and also included in the current proposal, see below in Table 10 and 10.10.2.

10.10.1 Adverse effects on sexual function and fertility

Table 9: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
Magnesium metaborate			
Combined repeated dose toxicity study with reproduction/developmental toxicity screening (oral gavage). OECD TG 422. GLP. Minor deviations considered not to impact the quality or outcome of the study. Rat/Sprague Dawley (Crl:CD(SD)), male/female 15 rats/sex/group for control and high dose, 10 rats/sex/group for other dose groups. The extra 5 males and 5 females in the control and high-dose groups were assigned to a 15- or 14-day recovery period, respectively. Reliability: 1 (by the registrant)	Magnesium metaborate. Purity not stated. Dose levels: 0, 15, 50, 125, 300 mg/kg/bw/day. Vehicle: arachis (peanut) oil. Duration of exposure: Males dosed for 28 days, females dosed from 14 days before pairing, through gestation until lactation day 13 (total 49-54 days). 5 animals/sex in the control and high-dose group was subject to a 14-15 day non-dosing recovery period.	*(Note: the dossier submitter does not have access to full study report and therefore numbers, level of statistical significance and % of changes are lacking for some parameters)* **300 mg/kg bw/day** <u>Males</u> Testicular tubular degeneration (minimal to marked) and lower testicular weights (stat. sign. compared to control) were observed after 28 days. After recovery, testicular findings were more pronounced with marked to severe tubular degeneration/ atrophy in all males. Lower epididymal weights (stat. sign. compared to control) and reduced epididymal luminal sperm and degenerate germ cells was observed in this group with more pronounced changes after recovery. Lower mean body weight gains throughout the study resulting in statistically significant lower mean body weights (7.4% to 13.7%) than the control group from study days 13 through 27 <u>Females</u> Longer mean gestation length (22.5 days) compared to the control group (21.3 days) ($p<0.01$). 3 of the 6 delivering females had gestation lengths of 23 days and occurred in conjunction with effects on intrauterine and postnatal survival in this group. **125 mg/kg bw/day** <u>Females</u> Longer mean gestation length (22 days) compared to the control group (21.3 days) ($p<0.01$). No substance-related effects on reproductive performance were observed at any dose.	Study report, 2017a More details in study summary in Annex I
13-Day oral (gavage) toxicity study in rats Non-guideline study and non-GLP. Range finding study for the OECD 422 study. Rat/Sprague Dawley (Crl:CD(SD)), male/female 5 rats/sex/group	Magnesium metaborate. Purity not stated. Dose levels: 0, 100, 300, 500, 1000 mg/kg/bw/day. Vehicle: arachis (peanut) oil.	At 1000 mg/kg bw/day all males and females were found dead or euthanized in extremis or by study day 5. At 500 mg/kg bw/day, one male was euthanized in extremis. All other animals at 500, 300 and 100 mg/kg bw/day survived until day 13. Macroscopic findings indicated no effects on sexual organs (testes, epididymides, ovaries with oviducts) in any of the dose groups. Microscopic examination was not performed.	Study report, 2017b

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
Reliability: 1 (by the registrant)	One daily dosing for 13 days.		
Boric acid			
Sub-chronic oral toxicity (90-day study) (<u>Study 1 and 2</u>) <u>Study 1:</u> No guideline specified Rat (Sprague-Dawley) male/female n = 10/sex/dose group <u>Study 2</u>: No guideline specified Dogs (Beagle) male/female n = 5/sex/dose group 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. The tissues preserved in buffered formalin and studied histopathologically 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. **Reproduction study (<u>Study 3</u>)** No guideline specified, but conforms to the standard three-generation, 2 litters per generation multi-generation studies normally used at the time. The high dose group P1 animals were sterile so only controls, low and mid-dose	**For studies 1 and 2:** Test material: boric acid or borax Purity: unknown <u>Doses/conc.:</u> -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 -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 <u>Exposure</u>: 90 consecutive days prior to necropsy (daily in feed). **For study 3:** Test material: boric acid or borax Purity: unknown <u>Doses/conc.</u>: 0, 117, 350 and 1170 ppm	**<u>Study 1 sub-chronic oral toxicity (rats):</u>** **52.5 ppm boron (equivalent to 4.7 mg B/kg bw/day):** One male and one female died during the study. <u>Males</u>: no changes in organ weights <u>Females</u>: non-statistically significant increased ovary weight (data not shown). **175 ppm boron (equivalent to 15.7 mg B/kg bw/day):** No statistically significant changes in growth, body weight, food consumption and organ weights for both males and females. **525 ppm boron (equivalent to 47.2 mg B/kg bw/day):** <u>Males</u>: partial testes atrophy (5 rats) and spermatogenic arrest (1 rat). <u>Females</u>: organ weights comparable to those of control (data not shown). **1750 ppm boron (equivalent to 157.5 mg B/kg bw/day):** One male and one female died during the study. <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. <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). **5250 ppm boron (equivalent to 472.5 mg B/kg bw/day):** 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). **<u>Study 2 sub-chronic oral toxicity (dogs):</u>** **17.5 ppm boron (equivalent to 0.4 mg B/kg bw/day):** <u>Males</u>: decreased spleen/body weight ratio (not specified if statistically significant, data not shown) <u>Females</u>: no reported changes in organ weights or organ/body weight ratios. **175 ppm boron (equivalent to 4.3 mg B/kg bw/day):** <u>Males</u>: decrease in testes/body weight ratio (not specified if statistically significant, data not shown) <u>Females</u>: no decrease in organ weight or organ/body weight ratios.	Weir and Fisher 1972 Weir 1966

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group[4]</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>groups were taken to the F2 and F3 generations.

Rat (Sprague-Dawley) male/female

n = 8 males/dose group and 16 females/dose group

Reliability: 2

Two-year feeding study (<u>Study 4)</u>

No guideline specified

Rat (Sprague-Dawley) male/female

n = 35/sex/dose group with 70/sex/dose group as controls</td>
<td>boron, equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw/day.

<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).

For study 4:

Test material: boric acid

Purity: unknown

<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.

<u>Exposure</u>: 24 months, daily in feed.</td>
<td>1750 ppm boron (equivalent to 43.7 mg B/kg bw/day):
One male dog died at day 68 of the study.
<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).
<u>Females</u>: increased width of the zona glomerulosa of the adrenal glands; markedly atrophied thyroid glands with lymphoid tissue infiltrations for 2 females.

<u>Study 3 reproductive toxicity (rats):</u>

For both low and mid-dose groups, no gross abnormalities for parents or offspring were reported.
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.

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="13">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>
<tr><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.
[b] Significantly higher than controls.

1170 ppm boron (equivalent to 58.5 mg B/kg bw/day):
All parent groups (P0) were found to be sterile. Only one female (1/16) produced a litter when mated with control males.</td>
<td></td>
</tr>
</table>

[04.01-MF-003.01]

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group[4]</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td><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).
<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).

<u>Study 4 two-year feeding study (rats):</u>
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.

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>Assessing the development of the boric acid-induced testicular lesions by light and electron microscopy

No guideline specified

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.

Rat (Fischer 344), male

n = 6/time-point (36 male rats in total) for administration of boric acid, and 5/time-point (30 male rats in total) as controls</td><td>Test material: boric acid

Purity: unknown

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.

Exposure: up to 4 weeks (in feed)

For the histology study and serum testosterone analysis, the animals were euthanised after 4, 7, 10, 14, 21 and 28 days of dosing.</td><td>After 4 days of exposure:
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.
1/6 male rat that presented severely disrupted spermatogenesis and no epididymal sperm, was not included in the analyses.

Up to 7 days of exposure:
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%).

Up to 10 days of exposure:
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%).

Up to 14 days of exposure:
Inhibited spermiation and peripheral spermatid nuclei</td><td>Treinen and Chapin 1991</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
Reliability: 2		(>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). **Up to 21 days of exposure:** 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 – 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). **At 28 days of exposure:** Over the 28-day study period, the rats consumed approx. 348.3 mg/kg/day boric acid (mean). 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%). **General toxicity** At day 28 the treated animals weighed 8% less (statistically significant, $p<0.05$) than the controls (controls = 288 g; boric acid = 265 g). No other signs of systemic toxicity were reported.	
Reproductive assessment by continuous breeding Performed according to the NTP's Reproductive Assessment by Continuous Breeding Protocol Mouse (Swiss) male/female n = 19/sex/dose groups 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 Purity: >99% 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.	**1000 ppm (equivalent to 26.6 mg B/kg):** 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 % for treated mice vs. 78 % for the controls), in 19/19 males. The histopathological exam did not reveal any significant changes for male mice; no histopathological results reported for F0 female mice. **4500 ppm (equivalent to 111.3 mg B/kg):** 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. In the male mice, the following statistically significant	Fail et al. 1991

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
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. Reliability: 2	Exposure: 27 weeks (daily in feed)	($p<0.05\%$) effects were reported, as compared to controls: - decreased mean sperm concentration (by approx. 72%); - decreased mean percentage of motile sperm (by approx. 32%); - increased mean percentage of abnormal sperm (by approx. 439%); - decreased seminiferous tubular diameter (by approx. 32%); - decreased number of spermatids in stages VII and VIII/tubule (by approx. 50%); - decreased spermatogenic index (by approx. 28%); - decreased absolute testis weight (by approx. 51%); - decreased absolute epididymis weight (by approx. 21%); - decreased prostate absolute weight (by approx. 20%). No statistically significant changes in body weight were observed. 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). **9000 ppm (equivalent to 221 mg B/kg):** F0: None of the F0 pairs was fertile. In the male mice, the following statistically significant ($p<0.05\%$) effects were reported, as compared to controls: - decreased mean sperm concentration (by approx. 95%), 12/15 males had no sperm; - decreased seminiferous tubular diameter (by approx. 63%); - no stage VII and VII spermatids/tubule (incidence not reported); - decreased number of spermatids/testis (x 10^4) by approx. 65%; - decreased absolute testis (by approx. 86%); - decreased absolute epididymis weights (by approx. 34%). 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). No histopathological results reported for F0 female mice. 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). **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	
Study investigating the	Test material:	**3000 ppm boric acid (equivalent to 26 mg B/kg**	Ku et al.

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
testicular toxicity of boric acid (BA) No guideline specified Rat (Fischer 344) male n = 6/dose group 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 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. 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. Reliability: 2	boric acid Purity: 99.99% 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. Exposure: 9 weeks (daily in feed)	**bw/day):** 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. **4500 ppm boric acid (equivalent to 38 mg B/kg bw/day):** 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: - a testis B level of 8 – 9 µg/g; - a variable increase in testicular spermatid head count (TSHC) (24% – 62% at week 2) and no statistically significant changes in testis weight; - a decrease in absolute epididymis weight (10% – 29%) and profound decrease in epididymal sperm count (ESC) (72% – 97%) during weeks 4 – 9. 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. **6000 ppm boric acid (equivalent to 52 mg B/kg bw/day):** 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: - a testis B level of 11 – 12 µg/g; - initially increased TSHC (31% – 51%) reflecting the inhibited spermiation at week 2; - progressive and profound decreases in absolute testis weight (12% – 68%) and TSHC (16% – 99%); - decreased absolute epididymis weight (12% - 57%) and decreased ESC (78% - 99%), reflecting the progression to testicular atrophy during weeks 3 – 9. No signs of post-treatment recovery from atrophy were observed. **9000 ppm boric acid (equivalent to 68 mg B/kg bw/day):** The adverse effects on male fertility at the highest dose level progressed similarly to the 6000 ppm dose level:	1993

Method, guideline, deviations if any, species, strain, sex, no/group[4]	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		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: - a testis B level of 15 – 16 µg/g; - initially increased TSHC (31% – 51%) reflecting the inhibited spermiation at week 2; - progressive and profound decreases in absolute testis weight (12% – 68%) and TSHC (16% – 99%); - decreased absolute epididymis weight (12% - 57%) and decreased ESC (78% - 99%), reflecting the progression to testicular atrophy by week 6. No signs of post-treatment recovery from atrophy were observed. **Feed consumption and body weight gain** At 68 mg B/kg bw/day, a decrease of 11% in feed consumption and a 16% reduced absolute body weight (270 g compared to 323 g in controls). No changes in body weight were observed for the other dose groups, and no other signs of general toxicity were reported.	
Assessment of the fertility of rats exposed to boric acid during spermatogenesis No guideline specified (conforms to Rodent Dominant Lethal Test) Rats (white outbred), n = 6 males/dose group 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	Test material: boric acid Purity: unknown 0, 1 and 10 mg B/kg bw/day Exposure: 60 days, daily oral gavage	No information on general toxicity was available for any of the dose groups. **1 mg B/kg bw /day** The fertility index was not different from control (86% versus 89% in controls). **10 mg B/kg bw/day** Reduced fertility index (62.5% compared to 89% in controls, unclear if statistically significantly different). Increased pre-implantation loss (23.81% compared to 2.69% in control, $p \leq 0.05$).	Marat et al. 2018

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
ovaries were investigated. 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.			
Sub-acute study No guideline specified Mouse (Swiss Albino) n = 10 males/dose group	Test material: Boric acid Purity: ≥99.5% 0, 115 (20.1), 250 (43.8), 450 (78.8) mg boric acid (B)/kg bw/day Exposure: 4-6 weeks via oral gavage	**After 4 weeks:** **≥115 mg boric acid/kg bw/day**: significantly ($p<0.001$) increased oxidative stress in sperm cells as observed by decreased membrane integrity **≥250 mg boric acid/kg bw/day**: significantly ($p<0.05$) increased MDA levels compared to control. 450 mg boric acid/kg bw/day: significantly ($p<0.05$) decreased GSH levels compared to control. **After 6 weeks**: **≥115 mg boric acid/kg bw/day**: significantly ($p<0.001$) increased oxidative stress in sperm cells as observed by decreased membrane integrity; significantly ($p<0.05$) decreased GSH levels and increased number of DNA damaged sperm cells and reduced cell viability in sperm cells. **≥250 mg boric acid/kg bw/day**: significantly ($p<0.05$) decreased sperm motility. **450 mg boric acid/kg bw/day**: significantly ($p<0.05$) increased MDA levels compared to control. In both groups (4 and 6 weeks): no differences found in testicular weight.	Aktas et al., 2020[5]
Borax			
Fertility assessment of male rats No guideline specified Rat (Sprague Dawley) male n = 18 males/dose group At the end of the 30 and 60	Test material: Borax (disodium tetraborate decahydrate) Purity: unknown <u>Doses/conc.:</u> 0, 500, 1000 and	**After 30 days of exposure:** **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. **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	Lee et al. 1978

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
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. Reliability: 2	2000 ppm borax, equivalent to 0, 50, 100 and 200 mg B/kg bw/day, respectively. Exposure: 30 and 60 days (daily in diet)	(incidence not reported). **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%). 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). 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. **After 60 days of exposure:** **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. **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. **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. Testicular histology examination 32 weeks post-treatment showed persistent germinal aplasia (incidence not reported). 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. 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	

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
		bw/day.	

[4] Where applicable and unless stated otherwise, the reliability scores of the studies presented in Table 9 are according to the CLH dossier of boric acid (2013), assessed by RAC in 2014.

[5] Adapted from CLH-report of trimethyl borate (ECHA, 2021d)

Table 5: 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
Boron				
Study type: cohort study (retrospective)	Boron, environmental and occupational exposure	Total population: 212 workers Low exposure group: DBE = 15.07 mg B/day, (74.03 ng B/g blood) Medium exposure group: DBE = 19.85 mg B/day, (126.6 ng B/g blood) High exposure group: DBE = 26.84 mg B/day, (269.2 ng B/g blood) Extreme exposure group: DBE = 47.17 mg B/day, (570.6 ng B/g blood, 571 ppb)	The study did not observe statistical significant differences in sperm quality parameters (concentration, morphology, motility) or reproductive hormone levels (LSH, FH and testosterone) between exposure groups.	Duydu et al., 2018a
Study type: cohort study (retrospective)	Boron, environmental and occupational exposure	Study in males in Bandirma and Bigadic in Turkey n: 212 Exposure groups based on boron blood levels. Very low exposure group (n: 12): <100 ng B/g blood Low exposure group (n: 17): 101-150 ng B/g blood Medium exposure group (n: 108): 151-450 ng B/blood High exposure group (n: 50): 451-600 ng B/g blood Overexposure group (n: 25): ≥651 ng B/g blood	No correlation between blood boron levels and DNA damage in sperm and lymphocytes. Statistically significantly lower (p = 0.042) micronucleus frequency observed in buccal cells in very low exposure group as compared to other exposure groups. However, sample size is low in the very low exposure group.	Basaran et al., 2019
Study type: cohort study (retrospective)	Boron, occupational and environmental exposure	Male workers in Bandirma and Bigadic, Turkey n: 304 Control group: <50 ng/g blood (DBE = 4.57 mg B/day) Low exposure group: 50-100 ng B/g blood (DBE = 8.32 mg/B/day) Medium exposure group:	Compared to control group, significantly (p<0.05) increased levels of boron found in semen and urine in medium, high and extreme exposure groups. No association between blood boron levels or semen boron levels and Y:X ratio in sperm.	Duydu et al., 2019[5]

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		100-150 ng B/g blood (DBE = 14.81 mg/B/day) High exposure group: 150-400 ng B/g blood (DBE = 23.50 mg B/day) Extreme exposure group: >400 ng B/g blood (DBE = 44.91 mg B/day) Daily exposure were determined by food/water sampling via double plate method	Furthermore, no significant effect observed on sex ratio at birth in groups exposed to boron vs. control group.	
Study type: cohort study (retrospective)	Boron, occupational and environmental exposure	Male workers employed in Bandırma, Turkey. Control group (n=77): 63.56 ng B/g blood Exposed group (n=86): 141.55 ng B/g blood	The mean blood boron concentration and mean semen boron concentration of the exposed group were significantly higher ($p<0.05$) than control group. The sperm concentrations or Y:X sperm ratios of workers were not affected. There was also no statistically significant correlation (Pearson, $p>0.05$) between blood/semen boron concentrations and Y:X sperm ratios in workers and no shift in the sex ratio at birth toward females was observed.	Yalcin et al., 2019

[5]Adapted from CLH report for trimetyl borate (ECHA 2021d)
DBE: daily boron exposure; FSH: follicle stimulating hormone; LH: luteinizing hormone

Table 6: 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

Animal data

Data on magnesium metaborate

Combined repeated dose toxicity study with reproduction/developmental toxicity screening test (OECD TG 422) of magnesium metaborate in the rat (Study report, 2017)

In an OECD TG 422 guideline study (GLP), magnesium metaborate was administered by oral gavage to groups of 10 male and 10 female Sprague Dawley (Crl:CD(SD) rats at the doses 15, 50, 150 and 300 mg/kg bw/day (described in more detail in Annex I to the CLH report). Males were dosed once daily for 28 days and females were dosed once daily for 14 days prior to pairing through lactation day 13 (for a total of 49 to 54 days). A control group of 10 males and 10 females were dosed with vehicle alone (arachis (peanut) oil). Two recovery groups (5 males and 5 females each) were treated with the high dose (300 mg/kg bw/day) or the vehicle alone and then maintained for 14-15 days without treatment. The recovery animals were not mated.

Reproductive organ weights and histopathology

At 300 mg/kg bw/day, males displayed small and soft testes of lower weights than in the control group. Histopathological examination showed testicular tubular degeneration (of similar severity in left and right testes), displayed as variable degeneration and loss of germ cells and multinucleated germ cell formation. Germ cell degeneration was characterized by shrunken, hypereosinophilic germ cells. Minimal severity grade was characterized by individual germ cell degeneration and segmental depletion primarily affecting spermatocytes and round spermatids; reduced elongating spermatids; and retained step 19 spermatids in late stage tubules. Mild severity grade was characterized by more pronounced germ cell depletion accompanied by variable germ cell disorganization and exfoliation. Moderate severity grade was characterized by widespread, moderate depletion of spermatocytes and round and elongating spermatids, and moderate germ cell disorganization and exfoliation. Marked severity grade was characterized by generalized depletion of germ cells with scattered Sertoli cell-only tubules. Spermatogonia were present in all severity grades. In addition, small epididymides of lower weights were observed at 300 mg/kg bw/day and correlated with reduced luminal sperm mixed with cellular debris, compatible with sloughed, degenerate germ cells.

In the 300 mg/kg bw/day recovery group, epididymal and testicular findings were more pronounced than in males at the primary necropsy. Marked to severe tubular degeneration/atrophy, characterized by a preponderance of tubules lined almost exclusively by Sertoli cells with variable numbers of sloughed, degenerate germ cells.

No significant changes of the female reproductive organs were observed.

Fertility, parturition and sexual function

No substance-related effects on reproductive performance were observed at any dose. All females in all groups were gravid. The mean numbers of days between pairing and coitus in the test substance-treated groups were similar to the control group value. The mean lengths of oestrous cycles in these groups were also similar to the control group value.

Significantly ($p<0.01$) longer mean gestation lengths were noted for the 125 and 300 mg/kg bw/day groups (22.0 and 22.5 days, respectively) compared to the control group (21.3 days). Four females at 300 mg/kg bw/day failed to deliver. At 300 mg/kg bw/day, a higher number of unaccounted-for implantation sites, and consequently, mean live litter size on PND 0 (2.8 pups) was observed when compared to the control group (13.6 pups). In the 300 mg/kg/day group, 3 of the 6 females that delivered had gestation lengths of 23 days and in conjunction with the test substance-related effects on intrauterine and postnatal survival in this group, the longer gestation length was attributed to the test substance. For the 125 mg/kg bw/day group, the gestation length was within the Charles River Ashland historical control data range (20.9 to 22.1 days) and in the absence of similar effects on pre- and postnatal survival, the effect at 125 mg/kg bw/day was not considered test substance-related. Mean gestation lengths in the 15 and 50 mg/kg bw/day groups were similar to those in the control group. No signs of dystocia were noted at any dosage level.

General toxicity

Clinical observations of clear and/or red material around the mouth and red material around the nose were noted for F0 males and females in the 50, 125, and 300 mg/kg bw/day groups approximately 1 hour following dose administration generally throughout the dosing period. Although these observations were considered test substance-related, they generally did not persist to the daily examinations or detailed physical examinations and were not considered adverse. No other dose-related clinical observations were made. One female in the control group was found dead on study day 17 which could be due to gavage error. All remaining F0 males and females survived to the scheduled necropsies.

At 300 mg/kg bw/day, males exhibited statistically significant lower mean body weight gains throughout the study resulting in statistically significant lower mean body weights (7.4% to 13.7%) than the control group from study days 13 through 27. A higher mean body weight gain was observed in the 300 mg/kg bw/day recovery group compared to the control but the mean body weights in this group remained 9.7% to 15.5% lower than the control group at the end of the study. No significant changes in body weight or body weight gain was observed in the other dose groups. In females, lower mean body weight gains were observed at 300 mg/kg bw/day during gestation. The differences from the control group were significant ($p<0.01$) during gestation days 7 to 11, 14 to 17, 17 to 20, and for the overall gestation period (days 0 to 20). Mean body weights were 4.0% to 21.7% lower than the control group during gestation; the differences were significant ($p<0.05$ or $p<0.01$) on gestation days 14, 17, and 20. Evaluation of mean body weights and body weight gains during lactation was precluded by euthanasia of females that delivered by lactation day 2.

In conclusion, test substance-related effects on male reproductive organs were noted at 300 mg/kg/day in absence of marked general toxicity. In addition, a test substance-related longer gestation length was noted at 300 mg/kg/day. There were no adverse clinical observations reported in the dams and the effects on maternal body weight and body weight gains during gestation could be attributed to the reported intrauterine death. Therefore, the DS considers that the longer gestation length at 300 mg/kg bw/day occurred in absence of marked general toxicity. The effect in males and females are relevant for classification.

Data on boric acid and borate salts

The assessment of adverse effects on sexual function and fertility of magnesium metaborate 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 magnesium metaborate and simple borates such as boric acid and borate salts will predominantly exist as undissociated boric acid. The toxicokinetic and toxicological properties of magnesium metaborate after oral exposure are therefore expected to be similar to those of boric acid and borate salts.

<u>90-day oral toxicity studies in rats and dogs, a three-generation reproduction study in rats and a 2-year oral toxicity study in rats (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 publicly 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.

In conclusion, the repeated dose toxicity studies in rats and dogs and the 3-generation reproductive toxicity study in rats clearly indicate testes as the main targets of toxicity of boric acid and impairment of fertility. Data from the 2 years feeding study with boric acid in rats also demonstrates effects on testis. The effects are relevant for classification.

<u>Continuous breeding reproductive toxicity study (boric acid) (Fail et al., 1991)</u>

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

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.

<u>Nine-week oral repeated dose toxicity study (boric acid) (Ku et al., 1993)</u>

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.

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.

28-and 42-day oral repeated dose toxicity study (boric acid) (Aktas et al., 2020)

Aktas et al. exposed 10 male Swiss Albino mice/group to 0, 115, 250 or 450 mg boric acid/kg bw/day for 4 or 6 weeks via gavage. In spermatozoa, membrane integrity and live cells were significantly ($p<0.001$) decreased upon exposure to ≥115 (20.1) mg boric acid (B)/kg bw/day for 6 weeks (LOAEL), see Table 12. Furthermore, motility of sperm cells was significantly ($p<0.05$) decreased at ≥250 (43.8) mg boric acid (B)/kg bw/day after 6 weeks. Statistically significantly ($p<0.05$) increased levels of malondialdehyde (MDA), a marker for oxidative stress, were measured at ≥250 and 450 mg/kg bw/day after a 4- or 6-week treatment, respectively. Reduced glutathione (GSH) levels were statistically significantly ($p<0.05$) decreased at 450 and ≥115 mg/kg bw/day after 4 and 6 weeks, respectively. This demonstrated that boric acid induced oxidative stress in testicular tissue. Increased ($p<0.05$) DNA damage in sperm cells was observed at ≥115 (20.1) mg boric acid (B)/kg bw/day for 6 weeks as measured by the alkaline comet assay. Although the findings suggest genotoxicity in sperm cells, the OECD TG 489 for in vivo alkaline comet assay currently does not recommend to assess DNA damage in mature germ cells because of high variable background levels in DNA damage (OECD, 2016).

Table 7: DNA damage, cell viability and motility in sperm cells after a 6-week exposure to boric acid[5]

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

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

[5] Adapted from the CLH-report of trimetyl borate (2021d)

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 boric acid and borate salts

According to CLP Annex I, paragraph 3.7.1.3, 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 boric acid and borate salts show evidence of adverse effects on sexual function and fertility, mainly expressed as:

1) Alterations to the female and male reproductive system

Females

In the non-guideline 90-day oral repeated dose toxicity study of boric acid and borax 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.

In the OECD TG 422 reproductive toxicity screening test of magnesium borate there were no evidence of alterations to the female reproductive system up the highest dose tested of 300 mg/kg bw/day.

Males

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, 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 in rats, 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 in rats (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.

In the OECD TG 422 reproductive toxicity screening test of magnesium borate similar test substance-related effects on male reproductive organs were noted at 300 mg/kg/day in absence of marked general toxicity. Small and soft testes of lower weights than in the control group as well as small epididymides of lower weights were observed that correlated with reduced luminal sperm mixed with cellular debris. Moreover, testicular tubular degeneration (of similar severity in left and right testes), loss of germ cells and multinucleated germ cell formation were reported.

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. These effects are relevant for classification purposes.

In the OECD TG 422 reproductive toxicity screening test of magnesium borate a test substance-related longer gestation length was noted at 300 mg/kg/day. No substance-related effects on reproductive performance were observed up to the highest dose tested 300 mg/kg bw/day.

Human data

No human data on adverse effects on sexual function and fertility of magnesium metaborate is available.

Data on boron compounds

Epidemiological studies investigating the effects of environmental and occupational boron exposure are available in the open literature. The studies published until March 2014 on the potential effects of boron on fertility were discussed in the RAC opinions on boric acid, disodium octaborate anhydrate and disodium octaborate tetrahydrate. The data consist of epidemiological studies of males exposed to boron environmentally and/or occupationally. The RAC concluded that the human studies show no clear evidence of adverse effects on male fertility by boron. The exposure to boron in these studies were well below the LOAELs for fertility reported from studies in animals. RAC pointed out that these epidemiologically studies had several study design limitations and should therefore be regarded as additional information.

Several studies have been published since March 2014, mainly investigating the occupational exposure to boron. In 2018, Duydu et al. (2018a) published a cross-sectional study evaluating the hormone levels and sperm parameters in male workers occupationally exposed to boron in Turkey. The authors found no association between blood boron levels and semen parameters or hormone levels (FSH, LH, FSH). The 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 study performed by Duydu et al. (2018a) has been assessed by RAC in the Opinion on barium diboron tetraoxide (2020), where it was concluded that even if the epidemiological data show no clear effects on fertility and sexual function, they are not considered to contradict the effects seen in animal studies. Moreover, there is no evidence that the effects observed in animals are not relevant to humans.

Investigation of Y:X sperm ratio in occupationally exposed workers (Yalcin et al. 2019; Duydu et al. 2019; Robbins et al. 2008)

A recent study assessing the association between boron exposure and Y:X chromosome ratio in men occupationally exposed in a boric acid production zone in Turkey was published (Yalcin et al. 2019). The aim of this study was to either refute or confirm the inverse association between the high level of boron exposure and the decrease in Y:X sperm ratio in men from China, in a similar study conducted by Robbins et al. (2008). The semen samples assessed for the purpose of this recent study were obtained within the scope of an earlier project ("Boron Project – I"; 2008 – 2010) and cryopreserved in liquid nitrogen. The total number of remaining samples was 163, out of which 86 were from workers assigned to the exposed group (i.e. working in the boric acid production facilities) and 77 from workers assigned to the control group (i.e. working in the steam power plant, energy supply unit, demineralised water plant, mechanical workshop etc.). The biological samples were analysed for B content through inductively coupled plasma mass spectrometry, while the Y:X sperm ratio was determined using fluorescence in situ hybridisation (FISH).

The mean blood boron concentrations of the exposed workers were stat. sign. higher than the controls (141.55 ± 80.43 vs. 63.56 ± 43.89 ng B/g blood, respectively; $p<0.05$). Similarly, the semen B levels of the exposed workers were stat. sign. higher than of the control group (1703.42 ± 1895.09 vs. 1127.78 ± 1713.96 ng B/g semen, respectively; $p<0.05$). These stat. sign. increases in both semen and blood B levels were brought forward by Yalcin and colleagues as an argument to support the high level of daily B exposure (DBE) for the workers assigned in the exposure group. However, no DBE levels for the 86 exposed workers were provided in the study. In the previous work, the exposed group was divided into low, medium and high exposure groups with DBE levels of 7.39 ± 3.97, 11.02 ± 4.61 and 14.45 ± 6.57 mg B/day, respectively (Duydu et al. 2011). Regarding the blood B levels of controls, it should be noted that the previous studies report levels below the limit of quantification (LOQ), i.e. 48.5 ng B/g blood (Duydu et al. 2011), whereas the blood B levels for the control group reported by Yalcin et al. (2019) are above the LOQ, i.e. 63.56 ± 43.89 ng B/g blood (see Table 13 below). The DBE levels seem to correlate with the blood B levels for both controls and exposed Turkish and Chinese workers. However, the blood B levels for controls and exposed groups seem to lead to significantly higher semen B concentrations in the Turkish workers, as compared to blood B levels of the Chinese workers that present approx. 3-fold increased levels (141.55 ± 80.43 vs. 515.4 ± 805.7 ng B/g blood for the exposed Turkish and Chinese workers, respectively; Table 13).

Yalcin and colleagues did not find a stat. sign. correlation (Pearson, $p>0.05$) between blood/semen B levels and Y:X sperm ratio in workers assigned to the exposed group, and no shift towards female babies at birth was observed (see Table 13). It was thus concluded by the authors that the presented results refute the positive association between high B exposure levels and decreased Y:X sperm ratios, as reported by Robbins et al. (2008).

However, the study conducted by Yalcin et al. (2019) presents several limitations which might have influenced the results. Firstly, even if the workers constituting the control group were not selected from boric acid and borate salts production areas, they were still exposed to B through drinking water from the central cafeteria and/or infirmary of the plant. The high B contamination (9.47 ± 0.18 mg B/L) of these water sources was not anticipated in the planning phase of the study and thus, this "background" exposure led to relatively high exposure of the control group. This is also reflected by the fact that the DBE levels for the Turkish control group were twice as high as for the Chinese control group that was not environmentally exposed (4.68 ± 1.63 vs. 2.3 ± 3.0 mg B/day; Table 13). Secondly, the exposure levels for the workers in the high exposure group were lower than the NOAEL set for male rat fertility. Assuming an average body weight of 70 kg, the high exposure group DBE levels can be converted to 0.2 ± 0.09 mg B/kg bw/day which is considerably lower than the NOAEL of 17.5 mg B/kg bw/day set for male rats.

Table 8: Characteristics of male workers assigned to the control and exposed groups

Number of participants	Mean age ± SD (years)	Mean duration of employment ± SD (years)	Mean total daily B exposure ± SD (mg B/day)	Mean blood B level ± SD (ng B/g blood)	Mean semen B level ± SD (ng B/g semen)	Mean Y:X sperm ratio ± SD (FISH)	Boys at birth (%)
Robbins et al. 2008 (China)							
n = 44 (controls)	31.3 ± 5.4	-	2.3 ± 3.0	45.5 ± 22.5	203.9 ± 105.7	0.99 ± 0.03	76.7
n = 39 (environmentally exposed)	30.0 ± 6.1	-	4.3 ± 3.1	109.11 ± 111.2	297.3 ± 273.0	0.96* ± 0.04	42.3
n = 63 (occupationally exposed)	31.2 ± 4.4	-	41.2 ± 37.4	515.4 ± 805.7	806.0 ± 612.6	0.93* ± 0.03	57.7
Yalcin et al. 2019 (Turkey)							
n = 77 (controls, however, environmentally exposed)	42.86 ± 5.06	18.02 ± 6.58	4.68 ± 1.63[#]	63.56 ± 43.89	1127.78 ± 1713.96	0.99 ± 0.03	48.5
n = 86 (occupationally and environmentally exposed)	42.45 ± 4.61	15.76 ± 7.16	7.39 ± 3.97 - 14.45 ± 6.57[#]	141.55 ± 80.43	1703.42 ± 1895.09	0.99 ± 0.02	54

FISH = Fluorescence in situ Hybridisation
* statistically significantly different from controls ($p<0.05$)
[#] the mean DBE levels were calculated and reported by the same authors in a previous publication (Duydu et al. 2011), where the group of exposed workers was further divided into low (DBE = 7.39 ± 3.97 mg B/day; n = 72), medium (DBE = 11.02 ± 4.61; n = 44) and high (DBE = 14.45 ± 6.57; n = 39) exposure groups.

Duydu et al. (2019) further investigated the Y:X chromosome sperm ratio in B-exposed workers from two boron mining facilities located in Bandirma and Bigadic, Turkey. Similarly, the semen samples assessed for the purpose of this study were obtained within the scope of earlier projects, i.e. “Boron Project – I” (2008 – 2010), “Boron project – II” (2014 – 2017), and cryopreserved in liquid nitrogen. A total of 304 biological samples (i.e. blood, semen and urine) were collected and analysed for B content and Y:X sperm ratio using mass spectrometry and FISH, respectively. Based on the blood B content, the workers were assigned into 5 different groups: controls (< 50 ng B/g blood), low exposure (> 50 – 100 ng B/g blood), medium exposure (> 100 – 150 ng B/g blood), high exposure (> 150 – 400 ng B/g blood) and extreme exposure groups (> 400 ng B/g blood) (see Table 14). The measured B semen levels were 36, 21, 12.4, 5.1 and 3 times higher than the blood B levels of the controls, low, medium, high and extreme exposure groups, respectively, which indicates that the male reproductive organs represent an accumulation site for B. Overall, the authors did not find a stat. sign. ($p>0.05$) association between B exposure and Y:X sperm ratios, the mean Y:X sperm ratios of the different exposure groups were not stat. sign. different in pairwise comparisons ($p>0.05$), and no B-associated shift in sex ratios at birth towards female offspring was seen. A negative association ($p < 0.05$) between reported pesticide application (information gathered through questionnaires) and Y:X sperm ratio for the total study group was seen.

However, the study presents several limitations that might have impacted the reported results. The different exposure groups were assigned based on blood B concentrations instead of DBE. This is reflected by the very high semen B levels measured in the workers assigned to the control group. The highest individual semen B value attributed to the control group exceeds the highest measured individual value from the extreme exposure group, i.e. 8597 vs. 8086 ng B/g semen, respectively. In addition, the control group was environmentally exposed to B through drinking water. It is important to note the mean semen B levels show a very large variation (e.g. 1598.46 ± 2027.85 ng B/g semen), including in the control group (i.e. 1077.11 ± 1845.34 ng B/g semen), therefore adding an extra layer of difficulty for identifying potential effects.

Moreover, based on an average body weight of 70 kg, the extreme DBE values calculated by this study will be 0.64 ± 0.26 mg B/kg bw/day, and the maximum individual DBE (i.e. 106.8 mg B/day) will be converted to 1.52 mg B/kg bw/day. As also indicated above, these values are considerably lower than the LOAEL for fertility in male 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 (ECHA, 2014a).

Table 9: Boron concentrations in biological fluids, DBE and other characteristics of male workers assigned to the control and exposed groups of workers

Number of participants	**Mean age ± SD (years)**	**Mean duration of employment ± SD (years)**	**Mean total daily B exposure (DBE) ± SD (mg B/day)**	**Mean blood B level ± SD (ng B/g blood)**	**Mean semen B level ± SD (ng B/g semen)**	**Mean Y:X sperm ratio ± SD (FISH)**	**Boys at birth (%)**
Duydu et al. 2019 (Turkey)							
n = 38 (controls, environmentally exposed)	42. 89 ± 5.32 (26 – 48)	18.20 ± 6.49 (2 – 26)	4.57 ± 1.69 (0.20 – 7.54)	30.00 ± 10.12 (16.23 – 49.23)	1077.11 ± 1845.34 (52 – 8597)	0.98 ± 0.03 (0.85 – 1.02)	53.73
n = 60 (low exposure)	41.50 ± 6.05 (23 – 49)	15.79 ± 7.47 (0.17 – 23)	8.32 ± 5.71 (2.56 – 35.61)	76.00 ± 15.22 (50.17 – 99.91)	1598.46 ± 2027.85 (111 - 8615)	0.99 ± 0.02 (0.89 – 1.04)	45.95
n = 50 (medium exposure)	40.22 ± 6.09 (27 – 48)	15. 74 ± 7.51 (1 – 25)	14.81 ± 9.99 (2.56 – 47.18)	122.88 ± 15.34 (101.28 – 149.84)	1526.93 ± 1265.36 (189 – 4897)	0.99 ± 0.02 (0.94 – 1.09)	52.94
n = 87 (high exposure)	37.26 ± 7.46 (22 – 53)	9.15 ± 6.42 (0.5 – 23)	23.50 ± 13.94 (3.32 – 55.10)	247.37 ± 71.32 (150.99 – 391.92)	1259.65 ± 1446.11 (100 – 10542)	0.99 ± 0.02 (0.86 – 1.03)	55.63
n = 69 (extreme exposure)	36.61 ± 6.68 (23 – 50)	6.65 ± 4.84 (1 – 26)	44.91 ± 18.32 (7.95 – 106.79)	553.83 ± 149.52 (401.62 – 1099.93)	1643.23 ± 965.44 (188 – 8086)	0.99 ± 0.02 (0.95 – 1.06)	53.57

FISH = Fluorescence in situ Hybridisation

Other studies (Basaran et al. 2019; Bolt et al. 2020)

The DNA damage in lymphocytes, sperm and buccal cells of occupationally (n = 102), occupationally and environmentally (n = 110) exposed male workers from Bandirma and Bigadic, respectively, was analysed through comet and micronucleus assays (Basaran et al. 2019). The biological samples were obtained within the scope of “Boron project – II” (2014 – 2017). As also reported above, based on their blood B levels, the 212 participants were assigned into 5 different exposure groups: very low exposure (< 100 ng B/g blood), low exposure (101 – 150 ng B/g blood), medium exposure (151 – 450 ng B/g blood), high exposure (451 – 650 ng B/g blood) and overexposure groups (> 651 ng B/g blood) (see Table 15 below). The DBE and blood B levels corresponding to the 5 different exposure groups were not given in this article. Demographic information as well as information on potential confounders (alcohol, smoking, pesticide exposure) was gathered through a questionnaire. However, it was not further detailed if these potential confounders may have affected the study results. No statistically significant increases in DNA damage in blood, sperm and buccal cells were observed between the B-exposed groups. No stat. sign. differences were found for neither alkaline nor neutral comet assay in the sperm cells. No correlations were seen between the measured blood B levels of the 5 different groups and tail intensity values of the sperm samples, lymphocyte samples, frequencies of micronucleus (MN), binucleated (BN), condensed chromatin (CC), karyorrhectic (KHC), karyolitic (KYL), pyknotic (PYC) and nuclear bud (NBUD) cells. Based upon these results, the authors concluded that extreme occupational exposure to B (i.e. > 651 ng b/g blood) does not induce DNA damage in lymphocytes, sperm or buccal cells. These results are in line with those reported previously by the same authors (Duydu et al. 2012; Basaran et al. 2012) and indicate that no statistically significantly increases in DNA-damage or changes on semen parameters were found in the B-exposed Turkish workers.

As also stated in the RAC Opinion on boric acid (2014a), the Turkish studies were initially set up based on the assumption that different occupational categories would give groups with quantitatively different exposure to B. However, high B concentrations in drinking water resulted in high exposure also in the controls (without occupational exposure). Therefore, participants were grouped according to blood concentrations of B rather than based on occupational exposure, and it is not clear how well these groups were matched. Moreover, the group sizes for the very low, low and overexposure groups were limited (i.e. n = 12, 17 and 25, respectively), thus leading to low statistical power.

Table 10: Comet assay results in sperm samples, lymphocytes and buccal cells according to the different exposure groups

<table>
<tr><th>Number of participants</th><th colspan="2">Mean tail intensity ± SD in sperm
(alkaline comet assay)
(%)</th><th colspan="2">Mean tail intensity ± SD in sperm
(neutral comet assay)
(%)</th><th colspan="3">Mean tail intensity ± SD in lymphocytes
(alkaline comet assay)
(%)</th><th colspan="2">Mean micronucleus frequencies in buccal cells ± SD
(micronucleus assay)</th></tr>
<tr><td colspan="10">Basaran et al. 2019 (Turkey)</td></tr>
<tr><td>n = 12
(very low exposure)</td><td colspan="2">5.37 ± 1.63
(3.1 – 8.42)</td><td colspan="2">6.31 ± 1.16
(5.13 – 8.49)</td><td colspan="3">6.0 ± 2.69
(2.82 – 11.95)</td><td colspan="2">3.54 ± 2.73*
(1 – 9)</td></tr>
<tr><td>n = 17
(low exposure)</td><td colspan="2">5.61 ± 1.2
(3.97 – 8.96)</td><td colspan="2">6.09 ± 1.1
(4.22 – 7.81)</td><td colspan="3">7.79 ± 5.18
(1.85 – 24.5)</td><td colspan="2">5.13 ± 4.69
(0 – 19)</td></tr>
<tr><td>n = 108
(medium exposure)</td><td colspan="2">6.03 ± 4.83
(2.6 – 49.71)</td><td colspan="2">6.23 ± 1.36
(3.95 – 13.68)</td><td colspan="3">7.5 ± 5.34
(1.64 – 27.47)</td><td colspan="2">4.32 ± 3.82
(0 – 19)</td></tr>
<tr><td>n = 50
(high exposure)</td><td colspan="2">5.55 ± 1.88
(2.81 – 13.73)</td><td colspan="2">6.16 ± 1.26
(4.12 – 9.66)</td><td colspan="3">8.7 ± 7.94
(1.38 – 36.0)</td><td colspan="2">4.56 ± 3.61
(0 – 16)</td></tr>
<tr><td>n = 25
(extreme exposure)</td><td colspan="2">5.36 ± 1.88
(3.04 – 12.32)</td><td colspan="2">5.71 ± 0.97
(4.24 – 8.4)</td><td colspan="3">5.04 ± 2.26
(0.65 – 10.08)</td><td colspan="2">4.06 ± 2.93*
(0 – 10)</td></tr>
<tr><td colspan="10">Correlations of blood B levels and genotoxicity parameters</td></tr>
<tr><td>Correlations between blood B level and:</td><td>Sperm DNA damage</td><td>Lymphocyte DNA damage</td><td>MN</td><td>BN</td><td>CC</td><td>KHC</td><td>KYL</td><td>PYC</td><td>NBUD</td></tr>
<tr><td>Pearson correlations</td><td>0.028</td><td>-0.024</td><td>0.023</td><td>-0.052</td><td>-0.156*</td><td>0.047</td><td>-0.045</td><td>0.058</td><td>0.023</td></tr>
</table>

*Statistically significant difference between groups ($p<0.05$); MN – micronucleus; BN – binucleated; CC – condensed chromatin; KHC – karyorrhectic; KYL – karyolitic; PYC – pyknotic; NBUD – nuclear bud.

A review paper on the effects of boron compounds on human reproduction was recently published (Bolt et al. 2020). The results of several reproductive toxicity studies in humans from Argentina, China and Turkey are detailed, discussed and the measured DBE levels are compared to the NOAELs for fertility and developmental toxicity established in rats (see Table 16 below). Based on these previously published epidemiological studies, Bolt and colleagues state that, compared to the B blood levels at the boron-related NOAELs for male fertility and for developmental toxicity in rats, the blood level means of the highest occupational exposure groups in China and in Turkey are lower by factors of > 4 and > 2, respectively. Part of the persons in the highest B exposure groups in China and in Turkey reach or exceed the experimental B blood levels at the NOAEL for developmental toxicity in rats. Part of the persons in the highest B exposure group in China reach or exceed the experimental B blood levels at the NOAEL for impaired male rat fertility. In this sense, the highest individual blood B level recorded from occupationally exposed workers from China is 3568.9 ng B/g blood, corresponding to a maximum individual DBE of 470 mg B/ day. The latter would thus correspond to a value of 6.7 mg B/kg bw/day if a 70 kg average body weight is assumed, that is considerably lower than the NOAEL for rat fertility of 17.5 mg B/kg bw/day. Moreover, the study conducted by Robbins et al. (2010) presents a series of limitations, such as the influence of different lifestyle factors, co-exposure to other minerals in relatively high concentrations (e.g. Mg) and fertility being assessed through questionnaires/interviews.

Table 11: Human and experimental exposure to boric acid/borate salts and associated blood boron levels

Human studies	Estimated DBE (mg/day)	Blood B levels (ng B/ g blood)
Bolt et al. 2020 (review)		
Turkey, ENV - High dose group I (Sayli et al. 1998; Korkmaz et al. 2007)	6.8 (1.8 – 2.3)	-
Argentina, ENV - Total cohort of mothers (Igra et al. 2016)	-	130* (0.73 – 610)*
Turkey, ENV + OCCUP - High exposure group (Tuccar et al. 1998)	14.5 (3.3 – 36)	220 (150 – 450)
Turkey, ENV - High exposure group (women) (Duydu et al. 2018b)	25 (10 – 58)	280 (152 – 980)
USA, OCCUP - High dust exposure group (Culver et al. 1994)	58	260 (up to max. 330)
China, OCCUP - High exposure group (Robbins et al. 2010; Scialli et al. 2010 - review)	37 (2.3 – 470)	500 (20 – 3600)
Turkey, OCCUP - Extreme exposure group (Duydu et al. 2019)	45 (8.0 – 200)	550 (400 – 2000)
NOAEL for male rat fertility (mg/kg bw/day) (Weir et al. 1972)	17.5	2300#
NOAEL for developmental toxicity in rats (mg/kg bw/day) (Price et al. 1996a)	9.6	1270

ENV = environmental exposure, OCCUP = occupational exposure;
*Assuming equal distribution of B between serum and blood cells;
#Calculated by Bolt et al. (2020)

Furthermore, Bolt and colleagues state that human B exposures, even in the highest exposed cohorts, are still too low to reach the blood concentrations in order to exert toxic effects on reproduction. Thus, under the most extreme occupational exposure reported, concentrations of B within the human body that are reprotoxic cannot be reached. The authors conclude that based on these epidemiological data, the current categorisation of inorganic boron compounds should be reconsidered. However, it should be kept in mind that no studies on effects on fertility and sexual function in humans are available at exposure and/or blood B levels corresponding to the animal LOAELs. Assuming a blood density of 1060 kg/m3 and taking into account the uncertainty factors for inter-species and intra-human variability (EFSA 2012), the LOAEL of 58.5 mg B/kg bw/day set for rat fertility would correspond to approx. 7360 ng B/g blood in humans; the highest individual blood B level recorded in human samples was 3568.9 ng B/g blood (Robbins et al. 2010). Furthermore, there are no available data indicating that boron toxicokinetics from animals would not be relevant for humans.

Finally, the available epidemiological studies showing no effects on fertility and semen parameters, FSH, LH and testosterone levels at DBE levels that were substantially below the LOAELs and even NOAELs from corresponding animal studies, do not contradict the experimental data showing clear effects of impaired fertility in male rats.

Conclusion on human data

The available epidemiological studies 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 mostly investigate male fertility. 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, extreme and overexposure groups in these studies were considerably lower than the NOAELs and LOAELs reported for rat fertility. No studies on effects on fertility and sexual function in humans are available at DBE levels corresponding to the animal LOAELs.

Hence, as was also highlighted by the RAC (Opinions on boric acid (2014a), disodium octaborate anhydrate (2014b), disodium octaborate tetraborate (2014c), on the revision of concentration limits for reproductive toxicity for seven borates (2019), and on barium diboron tetraoxide (2020)) it is concluded that the available human data on fertility and sexual function do not contradict the animal data. The human data are therefore considered as additional information.

Overall, the available human data do not contradict the experimental data seen across several species (mice, rats and dogs) and give no evidence to support that the effects seen in animals are not relevant for humans.

10.10.3 Comparison with the CLP criteria

Classification for reproductive toxicity following oral exposure is based on a weight of evidence determination using substance specific data and 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. In a OECD TG 422 reproductive toxicity screening study magnesium metaborate was reported to have adverse effects on male reproductive organs and to cause longer gestation-length. The findings on male reproductive organs in this study are in line with data of boric acid and borax, thus supporting that read-across from boric acid and borax is justified for reproductive toxicity. The animal data on effects on fertility of the borates included in the present proposal has previously been assessed by the RAC (RAC opinion on boric acid; disodium octaborate anhydrate and disodium octaborate tetrahydrate, 2014a,b,c, and RAC opinion on barium diboron tetraoxide, 2020). The additional study included in this assessment by Marat et al (2018) does not present any conclusive data and the findings do not contradict the data previously assessed by RAC. The RAC concluded that studies of reproductive toxicity and repeated dose toxicity studies in mice, rats and dogs clearly indicate that boron impairs fertility through an effect on the testes. The effects observed in the different species are similar in nature. Based on data from the 2-year feeding study with boric acid in rats, the LOAEL for fertility is 334 mg/kg bw/day, equal to 58.5 mg B/kg bw/day. This conclusion is supported by the similar study with disodium tetraborate decahydrate. There were no indications that the impaired fertility is secondary to other toxic effects. The new information by Aktas et al. (2020) suggest a mechanism of oxidative stress in testicular tissue.

In conclusion, a large body of evidence based on read-across data of boric acid and borate salts from animal studies showing adverse effects of boron on sexual function and fertility, together with substance specific data on magnesium metaborate fulfil the classification criteria for magnesium metaborate as **Repr. 1B, H360F**.

Classification in Repr. 1A is not appropriate as read-across human data on boric acid and borate salts do not provide clear evidence of adverse effects on sexual function and fertility at boron exposure levels that were well below the LOAELs from corresponding animal studies. 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 read-across data from boric acid and borate salts is considered to be clear and not only *some evidence from humans or experimental animals*.

Concentration limits

According to the current CLP guidance (v.5 July 2017), concentration limits for adverse effects on sexual function and fertility should be based on the lowest ED10. The RAC has previously concluded that the most sensitive effect of boric acid on sexual function and fertility is testicular atrophy in a toxicity study in rats (RAC opinions on boric acid, disodium octaborate anhydrate and disodium octaborate tetrahydrate, 2014a,b,c). There is no reason to reconsider this conclusion based on the human information published since 2014. The incidence of testicular atrophy at 24 months was 10%, 40% and 100% at doses corresponding to 5.9, 17.5 and 58.5 mg/kg bw/day boron. The incidence in control animals was 30% (Study report, 1966a). The same incidences were observed with disodium tetraborate decahydrate (Study report, 1966b). Hence, the ED10 corresponds to 17.5 mg B/kg bw/day (100 mg boric acid/kg bw/day). According to section 3.7.2.6.3 of the CLP Guidance, a substance with a 4 mg/kg bw/day < ED10 < 400 mg/kg bw/day belongs to the medium potency group. None of the modifying factors related to type or severity of effect, data availability, dose-response relationship, mode/mechanism of action, toxicokinetics or bioaccumulation applies for boric acid. Since boric acid has a harmonised classification for reproductive toxicity in category 1B (H360FD), the GCL of 0.3% would apply (Table 3.14 of the CLP guidance). Concentration limits for magnesium metaborate were derived in a similar way by correcting for the percentage of boron (calculations are available in Table 25). Magnesium metaborate fall within the range of the medium potency group for effects on fertility, which means that the GCL of 0.3% should apply. Similar to boric acid, the modifying factors described above does not apply for magnesium metaborate.

10.10.4 Adverse effects on development

There is one combined repeated dose toxicity study with reproduction/developmental toxicity screening (OECD TG 422) of magnesium metaborate available for reproductive toxicity in the registration and no read-across from boric acid and borate salts is applied. However, according to the dossier submitter, there is no scientific evidence to conclude that read-across from boric acid and borate salts to magnesium metaborate, similar to other inorganic borate salts, would not be justified for systemic endpoints. Therefore, for the purpose of classification, read-across from boric acid and borate salts is used together with the substance-specific information in a WoE determination.

With the exception of recent studies by Marat et al. 2018 and Pleus et al. 2018, 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. The newer studies were also included in the CLH-proposals of sodium per(oxo)borates (ECHA, 2021a,b,c) and of trimethyl borate (ECHA, 2021d) Two epidemiological studies regarding developmental effects by boron exposure has been published since 2014. These are presented in Table 18 and were also included and discussed in the CLH-proposal for revising concentration limits for reproductive toxicity of boric acid and a number of borates (ECHA, 2019), in the CLH proposal of barium diboron tetraoxide (ECHA, 2020) and in the CLH-proposals of sodium per(oxo)borates (ECHA, 2021a,b,c) and of trimethyl borate (ECHA, 2021d).

Table 12: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Magnesium metaborate			
Combined repeated dose toxicity study with reproduction/developmental toxicity screening (oral gavage). OECD Guideline	Magnesium metaborate. Purity not stated.	*(Note: the dossier submitter does not have access to full study report and therefore numbers, level of statistical significance and % of changes are lacking for some parameters)*	Study report, 2017a

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
422. GLP. Minor deviations considered not to impact the quality or outcome of the study. Rat/Sprague Dawley (Crl:CD(SD)), male/female 15 rats/sex/group for control and high dose, 10 rats/sex/group for other dose groups. Reliability score 1 (reliable without restriction) by the registrant.	Dose levels: 0, 15, 50, 125, 300 mg/kg bw/day. Vehicle: arachis (peanut) oil. Duration of exposure: Males dosed for 28 days, females dosed from 14 days before pairing, through gestation until lactation day 13 (total 49-54 days). 5 animals/sex in the control and high-dose group was subject to a 14-15 days recovery period.	**Maternal effects** Lower mean body weight gains were observed at 300 mg/kg bw/day during gestation. The differences from the control group was significant ($p<0.01$) during gestation days 7 to 11, 14 to 17, 17 to 20, and for the overall gestation period (days 0 to 20). Mean body weights were 4.0% to 21.7% lower than the control group during gestation; the differences were significant ($p<0.05$ or $p<0.01$) on gestation days 14, 17, and 20. **Effects on the offspring** A lower number of pups were born at 300 mg/kg bw/day (from the delivering 6 dams) with a mean live litter size of 2.8 pups as compared to 13.6 in the control group. At 125 mg/kg bw/day survival was slightly lower than the control group during PND 0 to 4. Although the values were within historical control range and the differences from the control group were not statistically significant, the lower postnatal survival was considered test substance related. No effect on postnatal survival was observed at 50 and 15 mg/kg bw/day. At necropsies of pups found dead at 300 mg/kg bw/day malformation detected included anasarca (2 pups), cleft palate (1 pup), hydrocephaly (2 pups), microphthalmia (3 pups), only 12 pairs of ribs (1 pup), heart malformation (1 pup) and sternoschisis (1 pup). In addition, developmental variations in fetuses at 300 mg/kg bw/day included renal papilla(e) not fully developed and/or distended ureter(s) and a major blood vessel variation No foetal malformations or developmental variations were noted for pups that were found dead at 15, 50, or 125 mg/kg/day. Evaluation of anogenital distance of the single pup surviving until PND 2 at 300 mg/kg bw/day showed that it was smaller (2.51 mm) than in the control group value (3.68 mm). The anogenital distances in the 15, 50, and 125 mg/kg/day groups were similar to the control group values.	
Boric acid and borax			
Prenatal Developmental Toxicity Study GLP-compliant Rat (Crl: CD VAF/Plus	Test material: boric acid Purity: 98% Doses/conc.: 0,	**Maternal effects** No maternal deaths occurred and no treatment-related clinical signs of toxicity were observed in the dams, at any dose level. Increasing dietary concentrations of boric acid were positively associated with whole blood boron concentrations	Price et al. 1996a Price et al. 1997

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
(Sprague Dawley)) n = groups of 14 -17 females/dose group/phase Reliability: 1 In phase I the dams were sacrificed on Day 20 for detailed foetal examination. 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. 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.	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) <u>Exposure phase I</u>: days 0 - 20 post mating (nominal in diet) <u>Exposure phase II</u>: days 0 - 20 post mating (nominal in diet), then on normal diet until termination on day 21 postpartum	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. **Effects on the offspring** **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). The following skeletal changes were observed: - 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); - 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); At the highest dose (25 mg B/kg bw/day), these changes were more pronounced. **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. 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). **LOAEL (developmental toxicity):** 13.3 mg B/kg bw/day, based on reduced foetal body weight and increased incidence of short rib XIII	
Equivalent or similar to OECD TG 414 (Prenatal Developmental Toxicity Study) GLP-compliant Rabbit (New Zealand White), female n = 30 pregnant female rabbits/ treatment group Reliability: 1 The females were sacrificed on GD 30 and the numbers of uterine implantations, resorptions, dead foetuses	Test material: boric acid Purity: unknown <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 <u>Exposure</u>: treatment on days 6 - 19 post-mating, via	**Maternal effects** 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. A high vaginal bleeding incidence was observed in the highest dose group, where 2 - 11 pregnant females/day bled between GD 19 - 30. 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. **Foetal effects** At 44 mg B/kg bw/day, a statistically significantly increased rate of resorptions per litter (89.9 %; 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). The incidence of skeletal malformations (i.e. cleft sternum,	Price et al. 1996b Heindel et al. 1994

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
and live foetuses were examined.	oral gavage	detached extra rib – lumbar 1, fused sternebrae and fused rib) was increased, but not statistically significantly, compared to controls (19, 22, 29 and 29% for the control, 11, 22 and 44 mg B/kg bw/day dose groups, respectively). 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. At 44 mg B/kg bw/day statistically significant increased incidences compared to control were seen, as follows: - interventricular septal defect in 57% foetuses (as compared to 0.6% in control); - enlarged aorta in 36% foetuses (as compared to 0 in control); - papillary muscle malformations in14% foetuses (as compared to in 3)% in control; - double outlet right ventricle (pulmonary artery and aorta both arising from the right ventricle) in 14% foetuses (as compared to 0 in control). **LOAEL (maternal toxicity):** 44 mg B/kg bw/day, based on reduced food intake, reduced body weight gain and abortions **LOAEL (developmental toxicity):** 44 mg B/kg bw/day, based on increased resorptions and cardiovascular malformations in surviving foetuses	
Prenatal developmental toxicity of boric acid in mice and rats GLP-compliant Cesarean-originated, barrier-sustained CWDl (ICR) VAF/Plus outbred Swiss albino (CD-l) mice Crl:CD BR VAF/:Plus outbred Sprague-Dawley (CD) rats n = 26 – 28 female mice or rats/dose group Reliability: 2	Test material: boric acid Purity: 98 – 99% **Rats:** Doses/conc.: 0, 0.1, 0.2 or 0.4 % 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 Exposure (daily in feed): GD 0 – 20 for the dose levels of 14 up to 58 mg	**Observed effects in rats** **Maternal effects** At 58 and 94 mg B/kg bw/day statistically significantly decreased body weight by 11% and by 35%, respectively, compared to controls **Foetal effects** At 94 mg B/kg bw/day statistically significantly increased prenatal mortality (36% resorptions/litter compared to 4% in the controls). Statistically significantly reduced average foetal body weight for all treated groups compared to controls: - 7% decrease at 14 mg B/kg bw/day; - 13 % decrease at 29 mg B/kg bw/day; - 37 % decrease at 58 mg B/kg bw/day; - 50 % decrease at 94 mg B/kg bw/day. Statistically significantly increased incidence of foetuses with visceral or external malformations for all dose groups compared to controls: - at 29 and 58 mg B/kg bw/day, incidences were 8% and 50%, respectively, compared to 2% in the control group. - at 94 mg B/kg bw/day, the incidence was 73% compared to 2.79% in the control group.	Heindel et al. 1992

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	B/kg bw/day; 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; **Mice:** Doses/conc.: 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 Exposure (daily in feed): GD 0 – 17	At 58 mg B/kg bw/day and 94 mg B/kg bw/day statistically significantly increased incidence (100%) of litters with 1 or more foetuses with a skeletal malformation (24/24 litters and 14/14 litters, respectively compared to their respective control groups, 4/28 and 2/14). Increased incidences of malformations: - malformations of the eyes at 94 mg B/kg bw/day (i.e. displaced eye in 7/136 foetuses and convoluted retina in 9/136 foetuses), compared to the control group (0/215); - enlarged lateral ventricles of the brain at 58 mg B/kg bw/day (in 21/386 foetuses) and at 94 mg B/kg bw/day (in 36/136 foetuses) compared to the respective control groups (0/431 and 0/215) - agenesis of rib XIII 58 mg B/kg bw/day (in 24/386 foetuses) and at 94 mg B/kg bw/day (in 17/136 foetuses), compared to the respective control groups (1/431 and 0/215). 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%). **LOAEL (developmental toxicity for rats):** 14 mg B/kg bw/day, based on statistically significantly reduced average foetal body weight **Observed effects in mice** **Maternal effects** At 175 mg B/kg bw/day, maternal body weight was statistically significantly reduced (by approx. 25%) during the treatment period. A dose-related increase in the incidence of renal tubular dilation was observed at microscopic examination. At 43 and 175 mg B/kg bw/day, ovarian cysts were seen in 1 dam of each dose group. **Foetal effects** At 175 mg B/kg bw/day, statistically significantly increased resorptions (approx. 19% per litter compared to 6% in controls). At 79 and 175 mg B/kg w/day statistically significantly reduced foetal body weights (by approx. 12% and 33%, respectively compared to controls). At 175 mg B/kg bw/day: - statistically significantly increased incidence (approx. 8%) of foetuses with malformations as compared to the control group (approx. 2%). - statistically significantly increased incidence of short rib	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>XIII (10/250 foetuses) compared to control group (0/311).
- agenesis of one or more vertebra (lumbar) in 3/250 foetuses compared to 1/311 in control group.

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)</td><td></td></tr>
<tr><td>Reproductive toxicity assessment study

No guideline specified, but conforms to the standard three-generation, 2 litters per generation multi-generation studies normally used at the time.

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.

Rat (Sprague-Dawley) male/female

n = 8 males/dose group and 16 females/dose group

Reliability: 2</td><td>Test material: boric acid or borax

Purity: unknown

Doses/conc.: 0, 117, 350 and 1170 ppm boron, equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw

Exposure: 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>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.
At 58.5 mg/kg bw/day there were no offspring produced from P1 animals.

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:
<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="13">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="6">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>Weir and Fisher 1972</td></tr>
<tr><td>Reproductive assessment by continuous breeding</td><td>Test material: boric acid</td><td>Maternal effects
9000 ppm (equivalent to 221 mg B/kg/day):</td><td>Fail et al. 1991</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Performed according to the NTP's Reproductive Assessment by Continuous Breeding Protocol Mouse (Swiss) male/female n = 19/sex/dose groups 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. Reliability: 2	Purity: >99% 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. Exposure: 27 weeks (daily in feed)	Statistically significantly decreased body weight (data not shown) **Effects on the offspring** **1000 ppm** (equivalent to 26.6 mg B/kg/day): ***F1 pups***: no statistically significant changes were observed. ***F2 pups***: statistically significantly ($p<0.05$) decreased adjusted live pup weight (by approx. 3% compared to control). **4500 ppm** (equivalent to 111.3 mg B/kg/day): ***F1 pups***: statistically significant decreased parameters compared to controls: - adjusted live pup weight by approx. 14%; - number of litters/pair by approx. 51%; - live birth index by approx. 11%. Only 1/19 F1 dams had 5 litters and all her pups in the 4th litter were born dead. **9000 ppm** (equivalent to 221 mg B/kg/day): ***F0:*** No litters were born to F0 animals.	
Assessment of embryonic or foetal death after treatment of male rats during spermatogenesis No guideline specified Rats (white outbred), male n = 6 males/dose group	Test material: boric acid Purity: unknown Doses/conc.: 0, 1 and 10 mg B/kg bw/day Exposure: 60 days, daily oral gavage 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	**1 mg B/kg bw/day** Statistical significant ($p\leq0.05$) changes compared to control were observed for the following parameters: - living embryos/female: 8 (controls: 9.71); - dead embryos/female: 1.3 (controls: 0.71); - post-implantation loss: 13.62 % (controls: 6.92 %) **10 mg B/kg bw/day** Statistically significant ($p\leq0.05$) changes compared to controls were observed for the following parameters: - living embryos/female: 6 (controls: 9.71); - dead embryos/female: 1.3 (controls: 0.71); - post-implantation loss: 18.0 % (controls: 6.92 %)	Marat et al. 2018

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	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.		
Prenatal developmental toxicity study OECD TG 414 Rat (Sprague-Dawley) n = 25 females/ dose group GLP not specified Reliability: 2	Boric acid (20% w/w, purity not stated) in cellulose insulation (CI) aerosols 0, 15, 90, 270 mg CI/m3, nose only, equivalent to 0.65 (0.11), 4.0 (0.69) and 11 (2.0) mg boric acid (B)/kg bw/day Exposure: GD 6-19, 6 h/day	**Maternal effects** No difference in bw between exposure groups. Damage to organs was observed at GD20. **4.0 (0.69) mg boric acid (B)/kg bw/day**: increase incidence gross lesions in lung or liver (64%* vs. 4% in control), increase incidence pale lungs (40%* vs. 0% in control), increase incidence mottled lungs (36%* vs. 4% in control). **11.0 (2.0) mg boric acid (B)/kg bw/day**: increase incidence gross lesions in lung or liver (76%* vs. 4% in control), increase incidence pale lungs (64%* vs. 0% in control). **Foetal effects** Mean fetal bw was reduced. **4.0 (0.69) mg boric acid (B)/kg bw/day**: reduction bw females (-6%*). **11.0 (2.0) mg boric acid (B)/kg bw/day**: reduction bw males (-6%*) and females (-7%*).	Pleus et al., 2018[5]

Table 18: 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
Mother-child cohort study (prospective, follow-up until 6 months of age)	Boron, environmental exposure via drinking water	n = 194 mothers, 120 infants residing in Northern Argentina Infant urine and whole blood were collected at the two follow-ups after birth (at 3 and 6 months). Infant weight, length and head circumference were measured at the two follow-ups after birth.	At 0 – 3 months: each doubling of B levels in infant urine was associated with a decrease in bodyweight of 141 g (p<0.05) and a decrease in infant head circumference of 0.39 cm (p<0.05). At 3 – 6 months: each doubling of B in infant urine was associated with a 200 g (p<0.05) in infant weight and decrease of 0.57 cm (p<0.05) in infant	Hjelm et al., 2019

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		This study is a follow-up of the same mother-child cohort as was investigated by Igra et al. 2016.	length.	
Epidemiological study (retrospective)	Boron, environmental exposure	Females residing in Marmara, Turkey. n: 190 Pregnancy outcomes (sex ratio, preterm birth, birth weights, congenital anomalies, abortions, miscarriage, stillbirth, early neonatal death, neonatal death and infant death) determined based on questionnaire. Boron blood levels at time of pregnancy were estimated from levels at time of study.	No boron-mediated differences on pregnancy outcomes were detected between exposure groups (low exposure n=143; medium exposure n=29 and high exposure n=27) Estimated blood boron levels ranged from 151.81 to 957.66 (mean 274.58) ng/g in the high exposure group.	Duydu et al., 2018b
Mother-child cohort study (prospective)	Boron, environmental exposure	Prospective study. Mother:child cohort in Northen Argentina. n: 194. 1-3 samples of serum, whole blood and urine was taken during pregnancy. Infant weight , length and head circumference was measured at birth.	Serum B > 80 µg/l were found to be inversely associated with birth length (B-0.69 cm, 95% CI:-1.4, p=0.043 per 100 µg/L serum B). No statistical significant associations between boron exposure and birth weight or head circumference were found.	Igra et al., 2016

Table 13: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No other relevant studies for the assessment of developmental toxicity were available				

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

Animal data

Data on magnesium metaborate

Combined repeated dose toxicity study with reproduction/developmental toxicity screening test (OECD TG 422) of magnesium metaborate in the rat (Study report, 2017)

In the OECD TG 422 study, described in more detail above (section 10.10.2 and in Annex I to the CLH report), 6 dams at 300 mg/kg bw/day gave birth, resulting in a lower mean live litter size of 2.8 pups as compared to 13.6 in the control group. The differences were statistically significant ($p<0.01$). The other 4 females in this group failed to deliver and had entirely resorbed litters. In addition, significantly ($p<0.05$) lower postnatal survival was noted in this group from PND 0 to 1 due to total litter loss by PND 2 of the 6 females that delivered. A test substance-related increase in the number of pups found dead and missing (presumed cannibalized) was noted PND 0 to 2 and PND 0 to 7 compared to the control group. Further evaluation of postnatal survival in the 300 mg/kg bw/day group was precluded by the death/euthanasia of the pups. Also at 125 mg/kg bw/day survival was slightly lower than the control group during PND 0 to 1, 1 to 4, and birth to PND 4. Although the values were within the Charles River Ashland historical control data range and the differences from the control group were not statistically significant, the slightly lower postnatal survival observed in the 125 mg/kg bw/day group was considered test substance-related due to the increase in the number of pups found dead and missing which was consistent with the substance-related effects observed at 300 mg/kg bw/day and considered to be a dose-response effect. No effect on postnatal survival was observed for the 50 and 15 mg/kg bw/day groups.

Evaluation of F1 body weights at 300 mg/kg bw/day was precluded by the death or euthanasia of the majority of pups on PND 0 or 1. The pup body weight of the single pup in the 300 mg/kg/day group that was alive on PND 1 was 29.4% lower than the control group. At 125 mg/kg bw/day mean pup birth weights (PND 1) were similar to the control group. However, substance-related lower mean F1 male and female pup body weight gains were noted compared to the control group during PND 1 to 4 and 4 to 7; the differences were generally significant ($p<0.05$). Mean body weight gains in this group were similar to the control group during PND 7 to 10 and 10 to 13. As a result of the lower body weight gains during the first week of the postnatal period, mean F1 male and female pup body weights in the 125 mg/kg/day group were lower (up to 15.0% and 13.5% for males and females, respectively) than the control group during PND 4 to 13; the greatest deficit was observed on PND 7. At 50 mg/kg bw/day mean F1 male and female pup body weight gains were similar to the control group during PND 1 to 4 followed by test substance-related lower mean body weight gains during PND 4 to 7; the differences were significant ($p<0.05$) compared to the control group during PND 4 to 7. The lower mean body weight gains observed at 50 mg/kg/day were not considered adverse because there were no test substance-related effects on postnatal survival noted at this dosage level. Mean F1 male and female pup body weights in the 50 mg/kg/day group were up to 13.2% and 10.9% lower for males and females, respectively, than the control during PND 4 to 13; the greatest deficit was observed on PND 7. At 15 mg/kg bw/day mean F1 male and female pup body weights and body weight changes during were unaffected by parental administration of the test substance.

At necropsies of pups found dead the following malformation were found at 300 mg/kg bw/day: 2 pups with anasarca, 1 pup with cleft palate, 2 pups with hydrocephaly, 3 pups with microphthalmia, 1 pup with only 12 pairs of ribs present, 1 had a right-sided aortic arch (the aortic arch and descending aorta coursed to the right of the vertebral column, right carotid and subclavian arteries arose independently from the aortic arch [no brachiocephalic arch]; left carotid and subclavian arteries arose from a common vessel from the aortic arch), and 1 pup had sternoschisis (sternal band nos. 2 through 4 not joined). In addition, fetal developmental variations observed in fetuses in the 300 mg/kg/day group included renal papilla(e) not fully developed and/or distended ureter(s) for 2 pups and a major blood vessel variation (right carotid and subclavian arteries arose independently from the aortic arch [no brachiocephalic trunk] for another pup. It is unclear to the dossier submitter how representative the numbers of pups with malformations or variations are since it is noted that pups were missing (presumed cannibalized) PND 0-7. No malformations or developmental variations were noted for pups that were found dead in the 15, 50, or 125 mg/kg/day groups. Necropsy findings for F1 pups that were euthanized on PND 13 were not suggestive of any association with maternal administration of the test substance according to the study summary in the disseminated REACH registration of magnesium metaborate. The dossier submitter does not have access to the full study report and therefore do not have data on individual pups, dams or litters.

Evaluation of anogenital distance in the 300 mg/kg/day group was precluded by the death or euthanasia of the majority of pups on PND 0 or 1. The anogenital distance (2.51 mm) of the single pup in this group that survived until PND 2 was smaller than the mean control group value (3.68 mm). The anogenital distances (absolute, relative to pup body weight, and relative to the cube root of pup body weight) in the 15, 50, and 125 mg/kg/day groups were similar to the control group values.

Evaluation of areolae/nipple anlagen in the 300 mg/kg/day group was precluded by the death/euthanasia of all pups in this group by PND 2. Areolae/nipple anlagen in the F1 male pups in the 15, 50, and 125 mg/kg/day groups was unaffected by parental administration of the test substance when evaluated on PND 13.

In conclusion, a statistically significant lower mean live litter size at 300 mg/kg bw/day compared to the control group was evident. A lower post-natal survival was observed at both 300 mg/kg bw/day and at 125 mg/kg bw/day and a substance-related lower mean male and female pup body weight and body weight gains were also seen in these two dose groups. At necropsies of pups found dead at 300 mg/kg bw/day malformations and variations were detected. The reported developmental toxicity is relevant for classification and not considered to be a secondary non-specific consequence of other toxic effects.

Data on boric acid and borate salts

The assessment of adverse effects on the development of the offspring of magnesium metaborate is based on 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 magnesium metaborate after oral exposure are therefore expected to be similar as boric acid and borate salts.

<u>Prenatal developmental toxicity in rats (Price et al., 1996a)</u>

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.

<u>Prenatal developmental toxicity studies in rabbits (Price et al., 1996b; Heindel et al., 1994)</u>

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.

Prenatal developmental toxicity study via inhalation in rat (Pleus et al., 2018)

Pleus et al. (2018) conducted a prenatal developmental toxicity study (OECD TG 414) of boric acid in a mixture of cellulose insulation (CI) as used as common building material. 25 dams (Sprague-Dawley rats) per group were exposed to 0.65 (0.11), 4.0 (0.69) and 11 (2.0) mg boric acid (B)/kg bw/day (equivalent to 0, 15, 270 mg/m^3 CI, nose only), 6 h/day, exposed GD 6-19. In dams, damage to lung and liver were noted. Statistically significantly increased incidence of gross lesions were found in lung and liver at 4 (0.69) and 11 (2.0) mg boric acid (B)/kg bw/day; 64% and 76%, respectively. Furthermore, statistically significantly increased incidence of pale and mottled lungs were observed at 4 (0.69) mg boric acid (B)/kg bw/day (40% and 36%, respectively) and 11 (2.0) mg boric acid (B)/kg bw/day (64% and 8%, respectively).

Mean foetal body weight was significantly ($p<0.05$) reduced at 4 (0.69) and 11 (2.0) mg boric acid (B)/kg bw/day; -5% and -7%, respectively. No other adverse developmental effects were found in foetuses, including no abnormalities found in skeletal development, in contrast to other studies.

Daily exposures to boron were much lower in this study as compared to other studies; the highest dose was 11 (2.0) mg boric acid (B)/kg bw/day while LOAEL for developmental abnormalities earlier published is 76 (13.3) mg boric acid (B)/kg bw/day. It is not clear from this study to what extent adverse effects observed were due to other content (80% w/w) in cellulose material used in this study. Therefore, this study is regarded to be of less relevance and considered as supportive information.

Conclusion on animal studies of boric acid and borate salts

The existing animal data for effects on development of boric acid an borates has previously been assessed by the RAC (RAC opinions on boric acid, disodium octaborate anhydrate and disodium octaborate tetrahydrate, 2014a,b,c). The conclusion of the RAC was that developmental toxicity (malformations) was clearly observed in studies in rats and rabbits, the rat being the most sensitive species, with an overall NOAEL of 9.6 mg B/kg bw/day. The LOAEL corresponds to 13.3 mg B/kg bw/day. Malformations consisted primarily of anomalies of the eyes, the central nervous system, the cardiovascular system, and the axial skeleton (Price *et al*., 1996a). The most common malformations were enlargement of lateral ventricles in the brain and agenesis or shortening of rib XIII. There were no indications that the developmental effects were secondary to other toxic effects. In addition, the RAC stated that the teratogenicity was possibly caused by an altered hox gene expression, caused by inhibition of histone deacetylases, a mechanism that is likely to be relevant also for humans.

According to CLP Annex I, paragraph 3.7.1.4, *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:

<u>1) Death of the developing organism</u>

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).

In the OECD TG 422 reproductive toxicity screening test of magnesium borate lower test-substance related post-natal survival was observed at both 125 mg/kg bw/day and 300 mg/kg bw/day. Statistically significant ($p<0.01$) lower mean live litter size of 2.8 pups at 300 mg/kg bw/day as compared to 13.6 in the control group was seen and significantly ($p<0.05$) lower postnatal survival from PND 0 to 1 due to total litter loss by PND 2 of the 6 females that delivered were also reported at 300 mg/kg/day. 4 females in this group failed to deliver and had entirely resorbed litters.

<u>2) Structural abnormality</u>

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.

In the OECD TG 422 reproductive toxicity screening test of magnesium borate at necropsies of pups found dead at 300 mg/kg bw/day malformations and variations were detected, including anasarca, cleft palate, hydrocephaly, microphthalmia, only 12 pairs of ribs, heart malformation and sternoschisis. In addition, developmental variations in fetuses at 300 mg/kg bw/day included renal papilla(e) not fully developed and/or distended ureter(s) and a major blood vessel variation.

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).

In the OECD TG 422 reproductive toxicity screening test of magnesium borate test substance-related lower mean male and female pup body weight and body weight gains were seen in both at 125 mg/kg bw/day 300 mg/kg bw/day.

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.

In the OECD TG 422 reproductive toxicity screening test of magnesium borate there were indications of neurodevelopmental effects at the highest dose tested 300 mg/kg bw/day where 2 pups showed hydrocephalus.

Human data

No human data of magnesium metaborate on adverse effects on the development of the offspring is available.

Data on boron compounds

Epidemiological studies on possible adverse pregnancy outcomes in female workers, or females environmentally exposed to boron via food or drinking water were not available in 2014, and such data was therefore not discussed in the 2014 RAC opinions on boric acid, disodium octaborate anhydrate and disodium octaborate tetrahydrate.

In 2016, Igra et al. has published a prospective mother-child cohort study investigating environmental exposure of boron through drinking water on pregnant women from Argentina. A statistically significant inverse association was found between serum blood boron levels >80 µg/L and birth length (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). However, it cannot be excluded that the observed effects can be the result of a combined exposure to lithium.

In 2018, Duydu et al. (2018b) published a retrospective cohort study investigating birth weights of newborns and pregnancy outcomes of females environmentally exposed to boron via drinking water in Turkey. 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/day) was 1.3 µg B/g blood (Price et al. 1996a, 1997), whereas the mean blood boron concentration in the high exposure group from the epidemiological study was 0.27 µg B/g blood.

These two epidemiological studies have been assessed by RAC in the Opinion on barium diboron tetraoxide (2020). The RAC concluded that even if these studies show no clear effects on development of the offspring, there is no evidence that the effects observed in animals are not relevant to humans.

In 2019, Hjelm et al. have published a follow-up study of the mother-child cohort (n = 194) investigated previously by Igra et al. (2016). This study has at this point in time not been assessed by RAC but is included in the proposals for harmonised classifications of sodium per(oxo)borates (to be discussed in RAC 2022) by the dossier submitter.

In order to evaluate the potential impact of pre- and post-natal boron exposure on infant growth, samples of maternal drinking water, placenta, urine, whole blood and breast milk were collected. Both maternal and infant samples were analysed for arsenic and lithium that were also present in the drinking water. Boron concentrations in drinking water ranged between 377 – 16076 µg B/L (median: 5863 µg B/L; n = 114). As shown in Table 20, concentrations of B in maternal serum were similar to those in whole blood (third trimester, GW 28-39), both showing a moderate correlation with concentrations in drinking water (rs = 0.28; p = 0.0001). Maternal blood B levels markedly increased from late pregnancy, GW 33 on average (median value: 140 µg B/L, n = 78), to the first follow-up post-partum (median values: 263 µg B/L, n = 108). A strong correlation between B in cord blood and cord serum was also seen (rs = 0.82). The authors suggested that the high B concentrations in cord serum (median: 196 µg B/L, i.e. just in between the concentration in maternal serum in GW 33 and that at the first follow-up about 50 days post-partum) is indicative of a rapid transfer to the foetus. The correlation of B concentrations in cord blood with those in placenta (rs = 0.73; p < 0.001) was stronger than the correlation with concentrations in maternal blood at GW 33 (rs = 0.41; p < 0.001). Boron concentrations in breast milk (median: 274 µg/L at 0–3 months after delivery) were similar to and strongly correlated with those in maternal serum (median: 266 µg B/L; rs = 0.94). The correlation with arsenic and lithium in breast milk was rs = 0.49 and 0.64, respectively, but there was no association between the breast milk concentrations of boron and those of calcium, magnesium, phosphorous, zinc, iron and selenium (rs > 0.1).

Median birth weight was 3050 g and 8% of the infants had low birth weight (i.e. < 2500 g). In total, 76% of the infants were exclusively breastfed at the follow-up at 0-3 months and 57% at 3 – 6 months, as reported by the mothers. The correlation between B concentrations in infant urine collected at 0 – 3 months after birth and breast milk became markedly stronger if restricted to infants who were reported to be exclusively breastfed (rs = 0.68; p < 0.001). The boron concentrations in urine of infants who were reported to be exclusively breastfed at 0 – 3 months (median: 541 µg B/L, n = 81) were approx. twice as high as those in the breast milk they received (median: 266 µg/L, collected within an hour of the infant urine sampling). An even bigger difference was found for the exclusively breastfed infants at 3 – 6 months (median urine: 1327 µg B/L, median breast milk: 293 µg B/L, n = 55). The authors suggested that the higher B concentrations in urine of the infants that were not exclusively breastfed demonstrate the strong impact of water intake on infant boron exposure; this was particularly evident at 3–6 months, when fewer infants were exclusively breastfed.

The authors used two cross-sectional analysis models, adjusting for infant age only (Model A) and for infant age and several other parameters, including lithium and arsenic concentrations in maternal blood and urine during pregnancy (Model B), for both follow-up periods (Table 20). A significant inverse association of B in infant urine with infant weight, at 0 – 3 months was observed (Model A). A non-stat. sign. tendency of shorter infants at higher B concentrations in cord blood was noticed after the 0 – 3 months follow-up (Model B; p = 0.08). At 0 – 3 months, adjusting for additional covariates (Model B) gave rise to a stronger inverse association of urinary B and infant body weight, and also the inverse association with head circumference became stat. significant (p < 0.05). Each 2-fold increase of B levels in infant urine was associated with a decrease in bodyweight of 141 g and a decrease in infant head circumference of 0.39 cm. Neither arsenic, nor lithium in infant urine was significant in the models. At the 3 – 6 months follow-up, each 2-fold increase of B concentrations in infant urine was associated with a decrease of 200 g in infant weight and a decrease of 0.57 cm in infant length (Model B). The study had a high participation rate (88%) and a prospective design with measurements of the infants at birth and two follow-ups during the first 6 months, but a small sample size. A limitation is the exposure to other metals, such as lithium, of the infants that also received drinking water. The concentrations of lithium were correlated with those of boron in the exposure biomarkers, and all exposures were lower in exclusively breastfed infants than in those also given drinking water. However, the measures of exposure to lithium (and arsenic) were generally not significant in the used statistical models (with and without metal adjustments). Previous studies correlated high altitude with low birth weight. Hjelm and colleagues underlined that even if the current study was performed in the Andes at 3100 – 4070 m above sea level, most of the mothers were of indigenous origin. The ancestors of these women lived in villages situated at high altitude in the Andes and this has resulted in adaptation to high altitude, including reproductive fitness.

In conclusion, the results of the study conducted by Hjelm et al. (2019) show a strong correlation between B in maternal serum and breast milk which indicates that exposure to B in early infancy was inversely associated with infant weight, length and head circumference during the first 6 months of life. These results are in line with the previously published findings of the same research group, showing that maternal serum B concentrations during pregnancy were associated with impaired foetal growth in the same mother-child cohort (Igra et al. 2016).

Table 14: Boron exposure markers prenatally and in early infancy

Perinatal exposure markers		**Median (range) boron concentrations (µg/L)**
Hjelm et al. 2019 (Argentina)		
Prenatal exposure markers (n = 78)	Maternal serum (last trimester)	134 (30 – 447)
	Maternal whole blood (last trimester)	140 (27 – 332)
	Placenta (µg/kg)	133 (1.1 – 605)
	Cord blood serum	196 (69 – 658)
	Cord whole blood	177 (29 – 600)
First follow-up (0 – 3 months after birth; n = 108)	Maternal serum	266 (47 – 624)
	Maternal whole blood	263 (66 – 750)
	Breast milk	274 (46 – 786)
	Infant urine*	689 (105 – 9200)
Second follow-up (3 – months after birth; n = 93)	Breast milk	293 (65 – 1386)
	Infant whole blood	127 (37 – 1351)
	Infant urine*	1784 (389 – 15068)

*Adjusted to mean osmolality (122 and 223 mOsm/kg at 0 – 3 and 3 – 6 months, respectively).

Table 15: Early life boron exposure and infant anthropometry (multivariable-adjusted linear regression analysis) as published by Hjelm et al. (2019)

Exposure as boron concentration (µg/L)	**Infant outcomes**					
	Weight (g)/log$_2$ B (µg/L) (95% CI)	**p-value**	**Length (cm) /log$_2$ B (µg/L) (95% CI)**	**p-value**	**Head circumference (cm) /log$_2$ B (µg/L) (95% CI)**	**p-value**
First follow-up (0 – 3 months)						

Exposure as boron concentration (µg/L)	Infant outcomes					
	Weight (g)/log_2 B (µg/L) (95% CI)	p-value	Length (cm) /log_2 B (µg/L) (95% CI)	p-value	Head circumference (cm) /log_2 B (µg/L) (95% CI)	p-value
Maternal serum blood (last trimester)	**n = 140/138**		**n = 140/131**		**n = 136/121**	
Model A[a]	-29 (-108:51)	0.477	-0,19 (-0.50; 0.12)	0.221	-0.05 (-0.23; 0.12)	0.545
Model B[b]	-30 (-100; 41)	0.405	-0.23 (-0.50;0.05)	0.103	-0.06 (-0.25; 0.12)	0.509
Cord blood	**n = 92/83**		**n = 92/80**		**n = 90/71**	
Model A[a]	-63 (-234; 108)	0.464	-0.46 (-1.0; 0.13)	0.126	0.06 (-0.35; 0.47)	0.765
Model B[b]	-77 (-223; 69)	0.297	-0.52 (-1.1; 0.07)	0.082	-0.16 (-0.56; 0.25)	0.447
Infant urine (0 – 3 months)	**n = 113/112**		**n = 113/109**		**n = 113/100**	
Model A[a]	-83 (-158; -8.1)	0.030	0.04 (-0.26; 0.34)	0.798	-0.01 (-0.20; 0.19)	0.943
Model B[b]	-141 (-240; -42)	0.006	-0.07 (-0.53; 0.40)	0.773	-0.39 (-0.74; -0.04)	0.028
Second follow-up (3 – 6 months)						
Infant urine (0 – 3 months)	**n = 111/109/109**		**n = 111/106/106**		**n = 106/93/93**	
Model A[a]	-94 (-197; 8.5)	0.072	- 0.00 (-0.31; 0.31)	0.988	-0.04 (-0.22; 0.14)	0.665
Model B[c]	-200 (-377; -23)	0.027	-0.57 (-1.1; -0.03)	0.040	-0.30 (-0.64; 0.04)	0.083
Model C[d]	-176 (-343; -8.9)	0.039	-0.66 (-1.2; -0.11)	0.019	-0.23 (-0.52; 0.06)	0.125
Infant urine (3 – 6 months)	**n = 112/107**		**n = 112/101**		**n = 112/94**	
Model A[a]	-111 (-229; 6.0)	0.063	-0.34 (-0.70; 0.01)	0.059	-0.12 (-0.31; 0.08)	0.231
Model B[c]	60 (-154; 273)	0.580	-0.48 (-1.2; 0.26)	0.202	-0.21 (-0.62; 0.19)	0.304
Infant whole blood (3 – 6 months)	**n = 106/92**		**n = 106/87**		**n = 106/82**	
Model A[a]	-51 (-180; 78)	0.436	-0.12 (-0.50; 0.26)	0.528	-0.12 (-0.32; 0.07)	0.217
Model B[c]	-34 (-190; 123)	0.667	-0.10 (-0.60; 0.40)	0.694	-0.14 (-0.43; 0.15)	0.330

a Adjusted for infant age (days).
b Adjusted for infant age, birth weight, length, head circumference, sex, mothers height (cm), exclusively breastfed (yes/no) and lithium concentrations (log_2 µg/L) in maternal whole blood during pregnancy or infant urine, and arsenic concentrations (log_2 µg/L) in maternal urine during pregnancy or infant urine.
c Adjusted for infant age, birth weight, length, head circumference, sex, mothers height (cm), exclusively breastfed (yes/no) at time of exposure measurement, lithium concentrations (log_2 µg/L) in infant urine and arsenic concentrations (log_2 µg/L) in infant urine.
d As Model B[c], but adjusted for weight, length or head circumference at 0 – 3 months instead of at birth.

Conclusion on human data of boron compounds

The human data on developmental effects of boron should be seen as additional information for the assessment of human relevance of the observed developmental toxicity of boric acid and borate salts in animal studies in a weight of evidence assessment.

The retrospective study (Duydu et al. 2018b) reports no adverse effects on development at exposure levels that were well below the NOAEL for developmental effects in rats. The blood B levels for the women in the highest exposure group (mean value of 274.6 ng B/g blood, highest individual value was 957.7 ng B/g blood) were below those corresponding to the NOAEL for developmental effects in rats (i.e. 9.6 mg B/kg bw/day corresponding to 1270 ng B/g blood; Price et al. 1997). This study presents several limitations, mainly associated with the retrospective study design and small sample size.

The prospective study conducted by Igra et al. (2016) detected a dose-dependent influence on birth size at B exposure levels (that were below the NOAEL for developmental effects in animal studies) but it could not be excluded that the results were influenced by co-exposure to lithium. The follow-up results of the same mother-child cohort published by the same research group provides the first evidence that exposure to B during early infancy (via breast milk and drinking water) may have a negative effect on post-natal growth up to 6 months of age (Hjelm et al. 2019). The lithium concentrations correlated with those of B in the assessed exposure biomarkers. However, it should be noted that adjusting for Li and As concentrations in maternal whole blood and infant urine resulted in a stronger inverse association of urinary B and infant body weight, the inverse association with infant head circumference becoming stat. significant at the first follow-up.

Assuming a blood density of 1060 g/L, the highest individual maternal serum B concentration of 624 µg/L measured at the first follow-up, would result in 589 ng B/g blood. This value is below the level of 1270 ng B/g blood that corresponds to the NOAEL for developmental effects in rats. However, the two prospective studies are the first to show developmental effects of perinatal environmental B exposure.

Overall, the available human data on boron do not contradict the experimental data coming from animal studies performed with boric acid and borax and give no evidence to support that the effects seen in animals are not relevant for humans. Moreover, the same conclusion was stated in RAC opinions (2014a and 2020) on boric acid and borate salts where experimental data across several species (mice, rats and rabbits) are available.

10.10.6 Comparison with the CLP criteria

Classification for reproductive toxicity following oral exposure is based on a weight of evidence determination using substance specific data and 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. In a OECD TG 422 reproductive toxicity screening study magnesium metaborate was reported to have adverse effects on the development of the offspring. The findings in this study are in line with data of boric acid and borax, thus supporting that read-across from boric acid and borax is justified for reproductive toxicity.

The animal data on effects on developmental toxicity of the borates has previously been assessed by the RAC (RAC opinion on boric acid; disodium octaborate anhydrate and disodium octaborate tetrahydrate, 2014a,b,c), except for the non-guideline study by Marat et al., 2018. The findings of post-implantation loss and foetal death in Marat et al are not in contradiction with findings in previous studies assessed by RAC. The RAC concluded that developmental toxicity (malformations) was clearly observed in studies in rats and rabbits, the rat being the most sensitive species, with an overall LOAEL corresponding to 13.3 mg B/kg bw/day. There were no indications that the developmental effects were secondary to other toxic effects. In addition, the RAC stated that the teratogenicity was possibly caused by an altered hox gene expression, caused by inhibition of histone deacetylases, a mechanism that is likely to be relevant also for humans.

In conclusion, based on read-across data of boric acid and borate salts from animal studies there are clear evidence of adverse effects on development of the offspring, and the classification criteria for **Repr. 1B, H360D** is therefore met for magnesium metaborate.

Classification in Repr. 1A is not appropriate as read-across human data on boric acid and borate salts do not provide clear evidence of adverse effects on development of the offspring at boron exposure levels that were well below the LOAELs from corresponding animal studies. 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 development of the offspring from existing read-across data from boric acid and borate salts is considered to be clear and not only *some evidence from humans or experimental animals*.

Concentration limits

According to the current CLP guidance (v.5 July 2017), concentration limits for adverse effects on development should be based on the lowest ED10. The RAC has previously concluded that the most sensitive effect on development by borates is the increased incidence of short rib XIII, considered a malformation (RAC opinions on boric acid, disodium octaborate anhydrate and disodium octaborate tetrahydrate, 2014a,b,c). The human information which has been published since 2014 gives no reason to challenge this conclusion. The fetal incidence of the short rib XIII malformation was 1.2 and 1.5% at the LOAEL (13.3 [76] mg B [boric acid]/kg bw/day) and the highest dose (25 [143] mg B [boric acid]/kg bw/day), respectively. As the incidences are low, it is not possible to derive an ED10. In this instance, the LOAEL should be used for setting the SCL according to the guidance. Boric acid belongs to the medium potency groups (4 mg/kg bw/day < ED10 (LOAEL) < 400 mg/kg bw/day). None of the modifying factors related to type or severity of effect, data availability, dose-response relationship, mode/mechanism of action, toxicokinetics or bioaccumulation applies. As boric acid has a harmonised classification for reproductive toxicity in category 1B (H360FD) according to the CLP guidance, the GCL of 0.3% would apply (Table 3.14 of the CLP guidance). Concentration limits were derived for magnesium metaborate from the same LOAEL and by correcting for the percentage of boron (calculations are available in Table 25). Magnesium metaborate fall within the range of the medium potency group for adverse effects on development, which means that the GCL of 0.3% should apply. Similar to boric acid, the modifying factors described above does not apply for magnesium metaborate.

10.10.7 Adverse effects on or via lactation

Table 16: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Combined repeated dose toxicity study with reproduction/developmental toxicity screening (oral gavage). OECD Guideline 422. GLP. Minor deviations considered not to impact the quality or outcome of the study. Rat/Sprague Dawley (Crl:CD(SD)), male/female 15 rats/sex/group for control and high dose, 10 rats/sex/group for other dose groups. Reliability score 1 (reliable without restriction) by the registrant	Magnesium metaborate. Purity not stated. Dose levels: 0, 15, 50, 125, 300 mg/kg/bw/day. Vehicle: arachis (peanut) oil. Duration of exposure: Males dosed for 28 days, females dosed from 14 days before pairing, through gestation until lactation day 13 (total 49-54 days). 5 animals/sex in the control and high-dose group was subject to a 14-15 days recovery period.	Lower body weight gains and body weights were observed during the lactational period. At 300 mg/kg bw/day evaluation of F1 body weights was precluded by the death or euthanasia of the majority of pups on PND 0 or 1. At 125 mg/kg bw/day substance-related lower mean F1 male and female pup body weight gains were noted during PND 1 to 4 and 4 to 7; the differences were generally significant ($p<0.05$). As a result of the lower body weight gains, mean male and female pup body weights in the 125 mg/kg/day group were lower (up to 15.0% and 13.5% for males and females, respectively) than the control with the greatest deficit observed on PND 7. At 50 mg/kg bw/day mean male and female pup body weight gains were lower during PND 4 to 7. Mean F1 male and female pup body weights in the 50 mg/kg/day group were up to 13.2% and 10.9% lower for males and females, respectively, than the control with the greatest deficit on PND 7. At 15 mg/kg bw/day mean F1 male and female pup body weights and body weight changes were unaffected.	Study report 2017a

Table 17: 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 18: 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

Animal data

Data on magnesium metaborate

Combined repeated dose toxicity study with reproduction/developmental toxicity screening test (OECD TG 422) of magnesium metaborate in the rat (Study report, 2017)

In the OECD TG 422 study, described in more detail above (section 10.9.2), lower body weight gains and body weights were observed during the lactational period. At 300 mg/kg bw/day evaluation of F1 body weights was precluded by the death or euthanasia of the majority of pups on PND 0 or 1. At 125 mg/kg bw/day substance-related lower mean F1 male and female pup body weight gains were noted during PND 1 to 4 and 4 to 7; the differences were generally significant ($p<0.05$). As a result of the lower body weight gains during the first week of the postnatal period, mean F1 male and female pup body weights in the 125 mg/kg/day group were lower (up to 15.0% and 13.5% for males and females, respectively) than the control group during PND 4 to 13; the greatest deficit was observed on PND 7. At 50 mg/kg bw/day: mean F1 male and female pup body weight gains were lower during PND 4 to 7. Mean F1 male and female pup body weights in the 50 mg/kg/day group were up to 13.2% and 10.9% lower for males and females, respectively, than the control during PND 4 to 13; the greatest deficit was observed on PND 7. At 15 mg/kg bw/day mean F1 male and female pup body weights and body weight changes were unaffected by parental administration of the test substance.

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 equivocal 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; Fail et al. 1991), 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.

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. During lactation and until PND 21, there were no effects on viability or growth of the offspring at any dose level.

Human data

No human data on the effects of magnesium metaborate on or via lactation was available. Since magnesium metaborate belongs to a group of borates that can be expected to generate boric acid upon hydrolysis, read-across of data from boric acid and borates is used.

Data on boric acid and borate salts

In the absence of relevant data, there are no indications that boron exposure through lactation has adverse effects. It should however be noted that numerous studies have shown that borates are absorbed from the gastrointestinal tract, as indicated by increased levels of boron in the blood, tissues or urine or by systemic toxic effects in exposed individuals or laboratory animals. In addition, boron compounds have been found in human breast milk (BfR, 2005), with reported (background) concentrations of approximately 4 µg B/L (Hunt et al., 2005, as reported in WHO, 2009) and in an experiment where 1–13 g of boric acid was given to lactating women 10–285 mg/l was found in milk (Moseman, 1994).

A recent epidemiological study found a strong correlation between boron in maternal serum (266 µg/L) and breast milk (274 µg/L), indicating that there is no regulation of boron in the mammary gland, but possible transfer by passive diffusion (Hjelm et al. 2019). Due to rapid excretion of boron in the urine, the boron levels of maternal serum and breast milk were reported to be only a fraction (approx. 5%) of those measured in the drinking water (5800 µg/L). The authors found that boron exposure (via breast milk and drinking water) had a continuous effect on infant growth (up to 6 months of age), being associated with statistically significant decreases in infant weight and length. However, it is not possible to distinguish between prenatal and postnatal exposure and the available data are not sufficient to conclude that boron is present in potentially toxic levels in breast milk.

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 is no human evidence indicating a hazard of magnesium metaborate or boron to babies during the lactation period. Human data shows that boron is transferred to breast milk, however, data are not sufficient to conclude that boron is present in potentially toxic levels in breast milk.

There is 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.

The dossier submitter therefore proposes no classification for adverse effects on or via lactation due to lack of data.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Classification of magnesium metaborate for adverse effects on sexual function and fertility; and adverse effects on development of the offspring is warranted: **Repr. 1B, H360 FD**.

Classification of magnesium metaborate for adverse effects on or via lactation is not warranted.

Specific concentration limits for adverse effects on sexual function and fertility; and adverse effects on development of the offspring are not considered justified since the estimated ED10 values adjusted for boron equivalents are within the medium potency group (4 mg/kg bw/day < ED10 /LOAEL < 400 mg/kg bw/day).

Table 25: Derivation of ED10 values and concentration limits for magnesium metaborate based on boron content

Substance	Molecular formula	Molecular weight (g/mol)	Conversion factor for equivalent dose of boron*	ED10 for fertility corrected for boron-content (mg/kg bw/day)**	LOAEL for development corrected for boron-content (mg/kg bw/day)***	Proposed generic concentration limit (GCL, % w/w), fertility	Proposed generic concentration limit (GCL, % w/w), development
Magnesium metaborate	B2MgO4	109.925	0.20	17.5/0.20=87.5	13.3/0.20=66.5	0.3	0.3

* Molecular weight of boron is 10.81 g/mol.
** Based on read-across from boric acid and borate salts, for which the ED10 for effects on sexual function and fertility was set at 17.5 mg B/kg bw/day.
*** Based on read-across from boric acid and borate salts, for which the LOAEL for effects on development was set at 13.3 mg B/kg bw/day

10.11 Specific target organ toxicity-single exposure

Not evaluated in this CLH proposal.

10.12 Specific target organ toxicity-repeated exposure

Not evaluated in this CLH proposal.

10.13 Aspiration hazard

Not evaluated in this CLH proposal.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this CLH proposal.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this CLH proposal.

13 ADDITIONAL LABELLING

Not evaluated in this CLH proposal.

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15 ANNEXES

Annex 1

Annex 2 – Confidential