CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical names:

a) ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$) [1]; ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$), calcined [2]

and

b) colemanite ($CaH(BO_2)_3.2H_2O$) [1]; boron calcium oxide ($B_6Ca_2O_{11}$), hydrate (1:5) [2]; colemanite, calcined [3]

and

c) tincalconite ($B_4Na_2O_7.5H_2O$)

EC Numbers:

a) - [1]; 296-662-5 [2]

b) - [1]; - [2]; 296-640-5 [3]

c) -

CAS Numbers:

a) 1319-33-1 [1]; 92908-33-3 [2]

b) 1318-33-8 [1]; 854267-07-5 [2]; 92908-12-8 [3]

c) 12045-88-4

Index Numbers:

a) **005-RST-VW-Y**

b) **005-RST-VW-Y**

c) **005-RST-VW-Y**

Index Number	Chemical names	EC Numbers	CAS Numbers
005-RST-VW-Y	ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$) [1]; ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$), calcined [2]	- [1]; 296-662-5 [2]	1319-33-1 [1]; 92908-33-3 [2]
005-RST-VW-Y	colemanite ($CaH(BO_2)_3.2H_2O$) [1]; boron calcium oxide ($B_6Ca_2O_{11}$), hydrate (1:5) [2]; colemanite, calcined [3];	- [1]; - [2]; 296-640-5 [3]	1318-33-8 [1]; 854267-07-5 [2]; 92908-12-8 [3]
005-RST-VW-Y	tincalconite ($B_4Na_2O_7.5H_2O$)	-	12045-88-4

Contact details for dossier submitter:

Swedish Chemicals Agency

Esplanaden 3A, P.O Box 2

SE-172 13 Sundbyberg, Sweden

kemi@kemi.se

+46 8 519 41 100

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CONTENTS

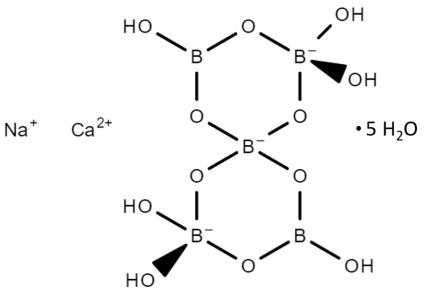
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substances

1.1.1 Ulexite (a)

Numerical identifiers, chemical identifiers and information related to molecular and structural formula of the borate mineral ulexite, including its calcined form, can be found in Table 1 and 2.

Table 1: Substance identity and information related to molecular and structural formula of ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$) [1]

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Sodium calcium pentaborate octahydrate
Other names (usual name, trade name, abbreviation)	Ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$) Ulexite
ISO common name (if available and appropriate)	Not available
EC number (if available and appropriate)	Not available
EC name (if available and appropriate)	Not available
CAS number (if available)	1319-33-1
Other identity code (if available)	Not available
Molecular formula	$B_5CaH_{16}NaO_{17}$
Structural formula	$NaCa[B_5O_6(OH)_6]\cdot 5H_2O$[1]: 
SMILES notation (if available)	[Na+].[Ca+2].OB1O[B-](O)(O)O[B-]2(O1)OB(O)O[B-](O)(O)O2.O.O.O.O.O
Molecular weight or molecular weight range	405.3 g/mol
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 relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

[1] The structure represents the actual arrangements of groups based on X-ray crystallography. However, the IUPAC name is based on the semi-empirical formula of ulexite: NaCaB5O9·8H2O. In the semi-empirical formula, the B-OH groups are accounted for as hypothetical water content.

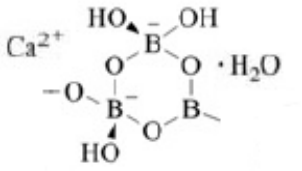
Table 2: Substance identity and information related to molecular and structural formula of ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$), calcined [2]

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Not available
Other names (usual name, trade name, abbreviation)	Ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$), calcined Ulexite, calcined
ISO common name (if available and appropriate)	Not available
EC number (if available and appropriate)	296-662-5
EC name (if available and appropriate)	Ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$), calcined
CAS number (if available)	92908-33-3
Other identity code (if available)	Not available
Molecular formula	Not available
Structural formula	Not available
SMILES notation (if available)	Not available
Molecular weight or molecular weight range	261.1 - 405.3 g/mol
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)	In the calcination process, solid ulexite (CAS number 1319-33-1) is heated whereby crystal water is lost from the structure. The degree of dehydration depends on the temperature applied and duration of heating. At about 400°C, complete dehydration is reached. The thermal treatment can also result in structural modifications due to the formation of micropores (Schubert, 2015; Sener and Ozbayoglu, 1992).
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

1.1.2 Colemanite (b)

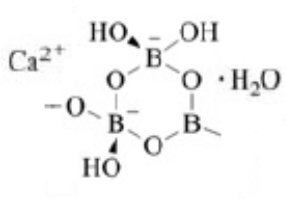
Numerical identifiers, chemical identifiers and information related to molecular and structural formula of the borate mineral colemanite, including its calcined form, can be found in Table 3 – 5.

Table 3: Substance identity and information related to molecular and structural formula of colemanite ($CaH(BO_2)_3.2H_2O$) [1]

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Dicalcium hexaborate pentahydrate
Other names (usual name, trade name, abbreviation)	Colemanite ($CaH(BO_2)_3.2H_2O$) Colemanite ($Ca_2B_6O_{11}.5H_2O$) Colemanite
ISO common name (if available and appropriate)	Not available
EC number (if available and appropriate)	Not available
EC name (if available and appropriate)	Not available
CAS number (if available)	1318-33-8
Other identity code (if available)	Not available
Molecular formula	$B_6Ca_2H_{10}O_{16}$
Structural formula	Structure of the repeating unit[2]: 
SMILES notation (if available)	Not applicable
Molecular weight or molecular weight range	411.1 g/mol
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 relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

[2] The structure of the repeating unit represents the actual arrangements of groups based on X-ray crystallography. However, the IUPAC name, molecular formula and molecular weight are based on the semi-empirical formula of colemanite: $Ca_2B_6O_{11}\cdot 5H_2O$. This equals two repeating units and the B-OH groups are accounted for as hypothetical water content.

Table 4: Substance identity and information related to molecular and structural formula of boron calcium oxide ($B_6Ca_2O_{11}$), hydrate (1:5) [2]

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Dicalcium hexaborate pentahydrate
Other names (usual name, trade name, abbreviation)	Boron calcium oxide ($B_6Ca_2O_{11}$), hydrate (1:5) Boron calcium oxide ($B_6Ca_2O_{11}$), pentahydrate Colemanite[2]
ISO common name (if available and appropriate)	Not available
EC number (if available and appropriate)	Not available
EC name (if available and appropriate)	Not available
CAS number (if available)	854267-07-5*
Other identity code (if available)	Not available
Molecular formula	$B_6Ca_2H_{10}O_{16}$
Structural formula	Structure of the repeating unit[3]: 
SMILES notation (if available)	Not applicable
Molecular weight or molecular weight range	411.1 g/mol
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 relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

*CAS number 12291-65-5 is frequently used by industry for colemanite. It has however been deleted from the CAS inventory and replaced by CAS number 854267-07-5.

[3] The structure of the repeating unit represents the actual arrangements of groups based on X-ray crystallography. However, the IUPAC name, molecular formula and molecular weight are based on the semi-empirical formula of colemanite: $Ca_2B_6O_{11}\cdot 5H_2O$. This equals two repeating units and the B-OH groups are accounted for as hypothetical water content.

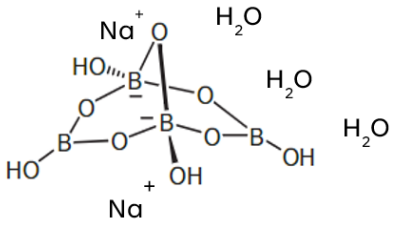
Table 5: Substance identity and information related to molecular and structural formula of colemanite, calcined [3]

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Not available
Other names (usual name, trade name, abbreviation)	Colemanite, calcined
ISO common name (if available and appropriate)	Not available
EC number (if available and appropriate)	296-640-5
EC name (if available and appropriate)	Colemanite, calcined
CAS number (if available)	92908-12-8
Other identity code (if available)	Not available
Molecular formula	Not available
Structural formula	Not available
SMILES notation (if available)	Not available
Molecular weight or molecular weight range	321.0 - 411.1 g/mol
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)	In the calcination process, solid colemanite (CAS number 1318-33-8/854267-07-5) is heated whereby crystal water is lost from the structure. The degree of dehydration depends on the temperature applied and duration of heating. At about 350 °C, complete dehydration is reached. The thermal treatment can also result in structural modifications due to the formation of micropores (Schubert, 2015; Sener and Ozbayoglu, 1992)
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

1.1.3 Tincalconite (c)

Numerical identifiers, chemical identifiers and information related to molecular and structural formula of the borate mineral tincalconite can be found in Table 6.

Table 6: Substance identity and information related to molecular and structural formula of tincalconite ($B_4Na_2O_7.5H_2O$)

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Disodium tetraborate pentahydrate
Other names (usual name, trade name, abbreviation)	Tincalconite ($B_4Na_2O_7.5H_2O$) Tincalconite Borax pentahydrate Boron sodium oxide ($B_4Na_2O_7$), pentahydrate
ISO common name (if available and appropriate)	Not available
EC number (if available and appropriate)	Not available
EC name (if available and appropriate)	Not available
CAS number (if available)	12045-88-4
Other identity code (if available)	Not available
Molecular formula	$B_4H_{10}Na_2O_{12}$
Structural formula	$Na_2[B_4O_5(OH)_4]\cdot 3H_2O$[4]: 
SMILES notation (if available)	[Na+].[Na+].OB1O[B-]2(O)OB(O)O[B-](O)(O1)O2.O.O.O
Molecular weight or molecular weight range	291.3 g/mol
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 relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

[4] The structure represents the actual arrangements of groups based on X-ray crystallography. However, the IUPAC name is based on the semi-empirical formula of tincalconite: $Na_2B4O_7\cdot 5H_2O$. In the semi-empirical formula, the B-OH groups are accounted for as hypothetical water content.

1.2 Composition of the substances

The constituents of the borate minerals included in the present CLH-proposal are given below (Table 7 – 24). There are no impurities or additives that affect the classification of the substances.

1.2.1 Ulexite (a)

Ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$) [1]

Table 7: 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)
Ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$) EC number: - CAS number: 1319-33-1	No information available	Not included in Annex VI	Eye Irrit. 2; H319 Repr. 1A; H360 Repr. 1B; H360

Table 8: 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
No information available				

Table 9: 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
No additives					

Ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$), calcined [2]

Table 10: 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)
Ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$), calcined EC number: 296-662-5 CAS number: 92908-33-3	No information available	Not included in Annex VI	Eye Irrit. 2; H319 Repr. 1A; H360

Table 11: 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
No information available				

Table 12: 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
No additives					

1.2.2 Colemanite (b)

Colemanite ($CaH(BO_2)_3.2H_2O$) [1]

Table 13: 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)
Colemanite ($CaH(BO_2)_3.2H_2O$) EC number: - CAS number: 1318-33-8	No information available	Not included in Annex VI	Eye Irrit. 2; H319 Repr. 2; H361 Repr. 2; H361fd STOT SE 1; H370 (lungs) (inhalation)

Table 14: 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
No information available				

Table 15: 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
No additives					

Boron calcium oxide ($B_6Ca_2O_{11}$), hydrate (1:5) [2]

Table 16: 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)
Boron calcium oxide ($B_6Ca_2O_{11}$), hydrate (1:5) EC number: - CAS number: 854267-07-5[5]	No information available	Not included in Annex VI	No information available

Table 17: 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
No information available				

Table 18: 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
No additives					

Colemanite, calcined [3]

Table 19: 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)
Colemanite, calcined EC number: 296-640-5 CAS number: 92908-12-8	No information available	Not included in Annex VI	No information available

Table 20: 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
No information available				

[5] CAS number 12291-65-5 is frequently used by industry for colemanite. It has however been deleted from the CAS inventory and replaced by CAS number 854267-07-5.

Table 21: 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
No additives					

1.2.3 Tincalconite (c)

Tincalconite ($B_4Na_2O_7.5H_2O$)

Table 22: 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)
Tincalconite ($B_4Na_2O_7.5H_2O$) EC number: - CAS number: 12045-88-4	No information available	Not included in Annex VI	No information available

Table 23: 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
No information available				

Table 24: 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
No additives					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 25a:

	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	005-RST-VW-Y	ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$) [1] ulexite ($CaNaH_{12}(BO_3)_5.2H_2O$), calcined [2]	- [1] 296-662-5 [2]	1319-33-1 [1] 92908-33-3 [2]	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 25b:

	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	005-RST-VW-Y	colemanite ($CaH(BO_2)_3.2H_2O$) [1] boron calcium oxide ($B_6Ca_2O_{11}$), hydrate (1:5) [2] colemanite, calcined [3]	- [1] - [2] 296-640-5 [3]	1318-33-8 [1] 854267-07-5 [2] 92908-12-8 [3]	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 25c:

	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	005-RST-VW-Y	tincalconite ($B_4Na_2O_7.5H_2O$)	-	12045-88-4	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 26: 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

The borate minerals included in the present CLH-proposal have not previously been discussed and/or agreed by the TC C&L (Dir. 67/548/EEC) and are not included in CLP Annex VI.

Boric acid and the several refined inorganic borate salts (e.g., borax, disodium octaborate and orthoboric acid, sodium salt) have a harmonized classification (CLH) as Repr.1B, H360FD. Some of them are also included in the Candidate list of substances of very high concern and have been recommended for inclusion in REACH Annex XIV (6th and 10th recommendation). The harmonised classifications of the refined inorganic borate salts have largely been based on read-across of toxicological data from boric acid and borax.

Regarding notifications to the C&L Inventory of ECHA, ulexite (CAS no 1319-33-1) is self-classified as Repr. 1A by 21 notifiers and Repr.1B by 1 notifier. Ulexite, calcined (CAS no 92908-33-3) is self-classified as Repr. 1A by 20 notifiers, but it is not classified by 2 notifiers. Also, colemanite (CAS no 1318-33-8) is self-classified as Repr. 2 by 92 notifiers. Tincalconite and colemanite, calcined (CAS no: 92908-12-8) has no notifiers in the C&L Inventory.

Ulexite (CAS no 1319-33-1) is included in the REACH Annex III Inventory, that is a list of substances compiled by ECHA which are predicted as likely to meet criteria for CMR category 1A or 1B properties. The information source for the inclusion of ulexite in this list is the Repr. 1B conclusion established by IMAP (Inventory Multi-tiered Assessment and Prioritisation), under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) from the Department of Health of the Australian Government. The assessment by IMAP included also colemanite (CAS no 1318-33-8) and tincalconite (CAS no 12045-88-4). Based on this assessment, ulexite, colemanite and tincalconite received mandatory classification as Repr. 1B in Australia.[6]

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

Ulexite, colemanite and tincalconite (also referred to as “borate minerals” in the text below) are considered to fulfil the criteria for classification as toxic to reproduction (Repr. 1B, H360FD). Therefore, harmonised classification is justified according to Article 36(1)(d) of the CLP Regulation.

The proposed classification and labelling of the borate minerals for reproductive toxicity is based on read-across from boric acid and borax. The read-across is justified because after oral exposure the borate minerals dissociate and result in the formation of boric acid as the main species at acidic and neutral pH. The suggested classification as Repr. 1B, H360FD is comparable to that of the other borates in Annex VI.

5 IDENTIFIED USES

Data on the specific uses of borate minerals in the EU are very limited since naturally occurring minerals are exempted from registration in REACH. Use data for the borate minerals in the present CLH-proposal has been gathered from literature and publicly available databases:

- Ceramics: Borates are essential ingredients in the production of frits (powdered glass) used by the ceramic industry in ceramic glazes and enamels. Colemanite and to a lesser extent ulexite are used for this application (Schubert and Steffee, 2010; Mazuna and Levitskii, 2008; U.S. Geological Survey, 2020), Glass: Borates are used as additives in glass to improve various properties. Colemanite and ulexite are used for some of these applications depending on the required quality (U.S. Geological Survey Minerals Yearbook – Boron, 2017; European Commission, 2020), Fertilizers: Borates are commonly used in fertilizers since boron is an essential micronutrient for plant growth, crop yield and seed development. Colemanite and ulexite are used for this application (Schubert, 2015; Abat, M. et

[6] Hazardous Chemical Information System (HCIS), http://hcis.safeworkaustralia.gov.au/HazardousChemical (accessed 30 January 2023)

al., 2015), Other uses: Besides the main applications for borate minerals as specified above, there are other uses. For example, tincalconite is used in impregnating agents for wood[7] and in pool sanitizers.[8]

6 DATA SOURCES

Information on ulexite, colemanite and tincalconite as well as read-across data included in the present CLH-report originates from study reports obtained from industry 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). Information on the borate minerals in the present CLH-proposal also originates from data in the scientific literature.

Additional relevant studies assessed in the CLH-proposals of sodium per(oxo)borates (ECHA, 2021a,b,c) and trimethyl borate (ECHA, 2021d) have also been included.

7 PHYSICOCHEMICAL PROPERTIES

Table 27: Summary of physicochemical properties of ulexite (CAS number 1319-33-1)

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid	Schubert, 2015	Observed
Melting/freezing point	1014 ºC	Gazulla, M.F. et al, 2005	Measured
Boiling point	-		
Relative density	1.96 g/cm^3	Schubert, 2015	Measured
Vapour pressure	-		
Surface tension	-		
Water solubility	7.6 g/l at 25 °C (0.49 wt% as $NaCaB_5O_9$)	Schubert, 2015	Measured
Partition coefficient n-octanol/water	-		
Flash point	-		
Flammability	-		
Explosive properties	-		
Self-ignition temperature	-		
Oxidising properties	-		
Granulometry	-		
Stability in organic solvents and identity of relevant degradation products	-		
Dissociation constant	pKa: 9.2 at 25 °C (boric acid)	Schubert, 2015	Measured for boric acid Read-across from boric acid is applied, as ulexite is converted into boric acid upon dissolution in water.

[7] https://www.woodsafe.se/en/content/woodsafe-pro

[8] https://www.poolwise.co.nz/?portfolio=ezi-chlor-trichlor-tetraborate

Property	Value	Reference	Comment (e.g. measured or estimated)
Viscosity	-		

Table 28: Summary of physicochemical properties of colemanite (CAS number 1318-33-8 / 854267-07-5)

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid	Schubert, 2015	Observed
Melting/freezing point	1051-1091 ºC	Gazulla, M.F. et al, 2005	Measured
Boiling point	-		
Relative density	2.42 g/cm^3	Schubert, 2015	Measured
Vapour pressure	-		
Surface tension	-		
Water solubility	a) 0.81 g/l at 25 °C b) 3.5 g/l at 25 °C (0.18 wt% as B_2O_3)	a) Safety data sheet, colemanite, Etimine, 2018[9] b) Schubert, 2015	Measured
Partition coefficient n-octanol/water	-		
Flash point	-		
Flammability	-		
Explosive properties	-		
Self-ignition temperature	-		
Oxidising properties	-		
Granulometry	-		
Stability in organic solvents and identity of relevant degradation products	-		
Dissociation constant	pKa: 9.2 at 25 °C (boric acid)	Schubert, 2015	Measured for boric acid Read-across from boric acid is applied, as colemanite is converted into boric acid upon dissolution in water.
Viscosity	-		

Table 29: Summary of physicochemical properties of tincalconite (CAS number 12045-88-4)

As tincalconite is the native form of borax pentahydrate (disodium tetraborate pentahydrate), read-across from physicochemical data in the REACH registration of borax (covering disodium tetraborate, anhydrous, disodium tetraborate pentahydrate and disodium tetraborate decahydrate) is applied where data on tincalconite is lacking.

[9] http://www.etimineusa.com/es/wp-content/uploads/2018/09/SDS-Colemanite-2016-2018_1.pdf

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid	Schubert, 2015	Observed
Melting/freezing point	> 1000 ºC (disodium tetraborate decahydrate)	REACH registration of disodium tetraborate, anhydrous (ECHA dissemination, 2023)	Measured for disodium tetraborate decahydrate
Boiling point	-		
Relative density	2.35 g/cm^3 at 26 ºC (disodium tetraborate, anhydrous)	REACH registration of disodium tetraborate, anhydrous (ECHA dissemination, 2023)	Measured for disodium tetraborate, anhydrous
Vapour pressure	-		
Surface tension	-		
Water solubility	49.74 g/L at pH 3.7 and 20 °C (disodium tetraborate, anhydrous)	REACH registration of disodium tetraborate, anhydrous (ECHA dissemination, 2023)	Measured for disodium tetraborate, anhydrous
Partition coefficient n-octanol/water	-		
Flash point	-		
Flammability	No ignition on contact with air (disodium tetraborate pentahydrate)	REACH registration of disodium tetraborate, anhydrous (ECHA dissemination, 2023)	Oberved for disodium tetraborate pentahydrate
Explosive properties	-		
Self-ignition temperature	No ignition on contact with air (disodium tetraborate pentahydrate)	REACH registration of disodium tetraborate, anhydrous (ECHA dissemination, 2023)	Observed for disodium tetraborate pentahydrate
Oxidising properties	-		
Granulometry	-		
Stability in organic solvents and identity of relevant degradation products	-		
Dissociation constant	pKa: 9.2 at 25 °C (boric acid)	Schubert, 2015	Measured for boric acid Read-across from boric acid is applied, as tincalconite is converted into boric acid upon dissolution in water.

Property	Value	Reference	Comment (e.g. measured or estimated)
Viscosity	-		

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this CLH-proposal

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 30: Summary table of toxicokinetic studies

Method	Results	Remarks[10]	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.	<u>*In vivo* dermal absorption</u>: 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 (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.	<u>Dermal absorption</u>: The absorbed doses of boric acid were 1.2 for 0.005 % dose, 0.28 for 0.5 % dose and 0.70 % for 5 % dose. These absorption amounts translated into flux values of 0.25, 0.58 and 14.58 mg/cm^2/h and permeability constants (Kp) of 5.0 x 10-4, 1.2 x 10^{-4} and 2.9 x 10^{-4} /cm/hr. The above doses were at a standard 1000 µL/cm^2 dosing solutions. When the 5 % dose was applied at 2 µL/cm2 (in vivo dosing volume), flux decreased some 200-fold to 0.07 mg/cm^2/hr and Kp of 1.4 x 10 $^{-6}$ cm/hr. Borax (disodium tetraborate decahydrate) dosed at 5 %/1000 µL/cm^2 had 0.41 % dose absorbed. Skin surface wash recovery was 87.7 ± 5.9 % dose. Flux was 8.5 µg/cm^2/h, and Kp was 1.7 x 10^{-4} cm/h. Disodium octaborate tetrahydrate dosed at 10	Test material: boric acid, disodium tetraborate decahydrate, disodium octaborate tetrahydrate Purity: unknown Reliability: 1	Wester et al. 1998b

[10] Where applicable and unless stated otherwise, the reliability scores of the studies presented in Table 30 are according to the CLH dossier of boric acid, assessed by RAC in 2014.

Method	Results	Remarks[10]	Reference
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.	% /1000 µL/cm^2 was 0.19 % dose absorbed. Skin surface wash recovery was 91.3 ± 25.2 % dose. Flux was 0.8 x 10^{-4} cm/h. 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).		
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	Absorption: inhaled boron is absorbed and systemically distributed, almost complete gastrointestinal absorption following oral exposure. Distribution: widely distributed throughout the body including reproductive tissues but has a low affinity for fat. At high doses, boron accumulates in the bone. Metabolism: being an inorganic element, boron is not metabolised by humans, but the parent borate is recovered in the blood, tissues and urine. Elimination and excretion: excretion primarily through renal elimination; over 93% of the inhaled and ingested dose is excreted in the urine; a calculated mean half-life of 13.4 h (range 4 – 27.8 h) in nine cases of boric acid poisoning.	The report considered human exposure to equivalent boron doses calculated from compounds such as boric acid, boron oxide, borate salts (e.g. calcium borate) and various hydration states of sodium borate salts (anhydrous, pentahydrate, decahydrate).	ATSDR Report, 2010
***In vivo* human excretion of boron, specifically examining renal clearance** 16 pregnant women in the 2nd trimester (14 – 28 weeks) and 15 nonpregnant	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	The source of boron used for the measurement of renal boron clearance was the dietary boron normally present	Pahl et al. 2001

Method	Results	Remarks[10]	Reference
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.	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. <u>Renal clearance for 24h period</u> 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. <u>Plasma levels</u>: 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.	in human food (present in high amounts especially in fruits and vegetables). Purity: unknown Reliability: 1	
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 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.	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. The upper limit for metabolic anomalies was > 7800 µg/L.	Environmental exposure to boron Reliability: 2	Minoia et al. 1990

Method	Results	Remarks[10]	Reference
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.			
Animal data			
Boric acid			
Rat (Sprague - Dawley), female n (renal clearance study) = 10 non-pregnant/group and 10 pregnant/group n (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[11]: 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, 6000 and 9000 ppm boric acid, equivalent to 0, 545, 788, 1050 and 1575 ppm boron (< 0, 0.2, 26, 38, 52, 68 mg B/kg bw/day), respectively.	Distribution: mean (± SD) testis B levels over the 9-week period were 5.6 ± 0.8, 8.8 ±0.7, 11.9 ± 1.4 and 15.1 ± 1.9 µg/g for 3000, 4500, 6000 and 9000 ppm boric acid, respectively. 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. Identified metabolites: none, boric acid is not metabolised.	Test material: boric acid Purity: 99.99% Reliability: 2	Ku et al. 1993

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

Method	Results	Remarks[10]	Reference
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 ± 0.05 (mean ± SE) at both days 4 and 7, excluding bone and adipose tissue. Days 4 - 7 (compared to controls): - bone showed a 37-fold increase (i.e., 16.37 ± 1.42 - 16.00 ± 0.71);	Test material: boric acid Purity: unknown Reliability: 2	Ku et al. 1991

Method	Results	Remarks[10]	Reference
	- 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. Identified metabolites: none, boric acid is not metabolised.		
Literature review of published and proprietary data	Absorption: oral absorption fraction in rats was found at 95%. Boron is readily absorbed through damaged skin in rabbits. Distribution: in male rats, boron is evenly distributed to liver, kidney, brain, muscle, adrenals, epididymis, testes, seminal vesicles, and blood, but not fat, following 61 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. Metabolism: being an inorganic element, boron is not metabolised by animals, but the parent borate is recovered in the blood, tissues and urine. Elimination and excretion: 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	Absorption: - 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 across intact skin for both animals and humans; for non-intact skin, the absorption varies with the used vehicle.	The review considered both human and experimental animal exposure to boric acid, which occurred through various routes of exposure (i.e.,	Murray 1998

Method	Results	Remarks[10]	Reference
	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).	oral, dermal, intravenous).	

Table 31: Other relevant studies

Method	Results	Remark	Reference
Ulexite			
In vitro boron (B) release study on ulexite ore with a particle size <100 µm 2h extraction in simple simulated gastric fluid at 37 °C and pH 1.5 for 2 hours (0.2 g/L loading) at an agitation speed of 100 revolutions per minute (rpm) for 1 hour followed by 1 hour of settling (without shaking). The extent of B release were based on the dissolved	An average value of 23.7 µg/L B (CVbetween-vessel = <0.5 %; N = 3) was found after 2 hours of extraction, corresponding with a boron release of 104 % (or 48 mg/m²)	The study was conducted according to the recommended Standard Operating Procedure (SOP) for Bioelution Testing of Metals, Inorganic Metal Compounds, and Metal-Containing Complex Materials: Simulated Gastric Fluid (Eurometaux, July 2016) which is based on ASTM D5517-07: Standard Test method for determining the extractability of metals	Study report, 2017a

boron concentrations obtained after 2 hours of extraction. To be noted, the test is in accordance with the draft new test guideline for 'Determination of relative metal/metalloid release using a simple simulated gastric fluid'.[12] However, according to the guideline two different loadings should be used: 0.2 g/L and 2 g/L. In the present study only 0.2 g/L was used.		from art materials - ASTM, 2007 (American Society for Testing and Materials).	
Colemanite			
In vitro boron (B) release study on colemanite ore with a particle size <100 µm 2h extraction in simple simulated gastric fluid at 37 °C and pH 1.5 for 2 hours (0.2 g/L loading) at an agitation speed of 100 revolutions per minute (rpm) for 1 hour followed by 1 hour of settling (without shaking). The extent of B release were based on the dissolved boron concentrations obtained after 2 hours of extraction. To be noted, the test is in accordance with the draft new test guideline for 'Determination of relative metal/metalloid release using a simple simulated gastric fluid'. However, according to the gudeline two different loadings should be used: 0.2 g/L and 2 g/L. In the present study only 0.2 g/L was used.	An average value of 25.2 mg/L B (CVbetween-vessel = 3 %; N = 3) was found after 2 hours of extraction, corresponding with a boron release of 103 % (or 45 mg/m²)	The study was conducted according to the recommended Standard Operating Procedure (SOP) for Bioelution Testing of Metals, Inorganic Metal Compounds, and Metal-Containing Complex Materials: Simulated Gastric Fluid (Eurometaux, July 2016) which is based on ASTM D5517-07: Standard Test method for determining the extractability of metals from art materials - ASTM, 2007 (American Society for Testing and Materials).	Study report, 2017b
Boric acid			
In vitro boron (B) release study on boric acid (particle size <100 µm) 2h extraction in simple simulated gastric fluid at 37 °C and pH 1.5 for 2 hours (0.2 g/L loading) at an	An average value of 36.5 mg/L B (CVbetween-vessel = 2 %; N = 3) was found after 2 hours of extraction, corresponding with a boron release of 107 %.	The study was conducted according to the recommended Standard Operating Procedure (SOP) for Bioelution Testing of Metals, Inorganic Metal Compounds, and Metal-Containing Complex	Study report, 2017c

[12] https://www.oecd.org/env/ehs/testing/draft-test-guideline-metal-release.pdf

agitation speed of 100 revolutions per minute (rpm) for 1 hour followed by 1 hour of settling (without shaking). The extent of B release were based on the dissolved boron concentrations obtained after 2 hours of extraction. To be noted, the test is in accordance with the draft new test guideline for 'Determination of relative metal/metalloid release using a simple simulated gastric fluid'. However, according to the gudeline two different loadings should be used: 0.2 g/L and 2 g/L. In the present study only 0.2 g/L was used.		Materials: Simulated Gastric Fluid (Eurometaux, July 2016) which is based on ASTM D5517-07: Standard Test method for determining the extractability of metals from art materials - ASTM, 2007 (American Society for Testing and Materials).	

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

There is no available substance specific information on the toxicokinetic or reproductive toxicity properties of the borate minerals assessed in this CLH proposal. Classification for reproductive toxicity following oral exposure is therefore based on a read-across approach from inorganic borates (borax or disodium tetraborate decahydrate) and boric acid, justified on the basis of their common behaviour in aqueous media.

The borate minerals in this CLH proposal are inorganic salts of boric acid and are described as moderately to very soluble in water. The solubilities of colemanite, ulexite and tincalconite in water at 25°C are 0.81 – 3.5 g/L, 7.6 g/L and 49.7 g/L, respectively.

In addition to the water solubility data of the borate minerals, in vitro tests on the release of boron at simulated physiological conditions have been made available to the DS for the purpose of harmonised classification. Boric acid and the borate minerals ulexite and colemanite were tested in simulated gastric fluid, at 37 °C and pH 1.5 for 2 hours (0.2 g/L loading) at an agitation speed of 100 revolutions per minute (rpm) for 1 hour followed by 1 hour of settling (without shaking). Measurements of the release of boron (in the form of boric acid in solution) were made after 2 hours of extraction. The results obtained are similar for all substances tested and show a complete availability of boron in simulated gastric fluid at the tested concentration, independent of test substance. These results indicate that the tested borate minerals and boric acid behave similar at simple simulated physiological conditions.

Following administration and prior to absorption into the systemic circulation, it is assumed that the borate minerals included in the present CLH-proposal 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 these borates contain only boric acid H_3BO_3, its conjugate base $B(OH)_4^-$ and the counter ions (Ca^{2+} and Na^+ for ulexite, Ca^{2+} for colemanite, and Na^+ for tincalconite). 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)[13], 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/liter equivalent basis when dissolved in water or biological fluids at the same pH and low concentration. Therefore, read-across to boric acid and borate salts for both toxicokinetic properties and systemic effects, based on boron (B) equivalents is justified. Conversion factors for the borate minerals comprised in the present dossier are given in Table 32.

Table 32: Overview of conversion factors of borate minerals to equivalent dose of boron

Substance	**Molecular formula**	**Conversion factor for equivalent dose of B**
Ulexite	$B_5CaH_{16}NaO_{17}$	0.13
Colemanite	$B_6Ca_2H_{10}O_{16}$	0.16
Tincalconite	$B_4H_{10}Na_2O_{12}$	0.15

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 boric acid or borate salts are available. The data described in Table 30 are mainly represented by what is available in the open scientific literature as experimental (animal data) and occupational studies, as well as 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.

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 in both humans and animals with the following common aspects:

- Boron is rapidly distributed throughout body fluids;

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

- 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 is 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 of 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 bw/hour in humans and 163 mg/kg bw/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 in the form of boric acid (up to 100%) into blood and distributed to tissues and organs. By dermal exposure, an uptake of 0.5% over intact skin has been suggested by the registrant(s) as a maximum value. Boric acid is not metabolized by the body and is excreted mainly via urine.

In aqueous solutions at physiological and acidic pH, low concentrations of ulexite, colemanite and tincalconite 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 ulexite, colemanite and tincalconite will therefore be expected to be similar to boric acid on a boron equivalents basis.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not assessed in this CLH-proposal.

10.2 Acute toxicity - dermal route

Not assessed in this CLH-proposal.

10.3 Acute toxicity - inhalation route

Not assessed in this CLH-proposal.

10.4 Skin corrosion/irritation

Not assessed in this CLH-proposal.

10.5 Serious eye damage/eye irritation

Not assessed in this CLH-proposal.

10.6 Respiratory sensitisation

Not assessed in this CLH-proposal.

10.7 Skin sensitisation

Not assessed in this CLH-proposal.

10.8 Germ cell mutagenicity

Not assessed in this CLH-proposal.

10.9 Carcinogenicity

Not assessed in this CLH-proposal.

10.10 Reproductive toxicity

The borate minerals included in the present CLH-proposal have no data on reproductive toxicity. For the purpose of harmonised classification, read-across from boric acid and borax is applied in the current proposal. The justification is based on their common behaviour in aqueous media, as described in section 9.1.

10.10.1 Adverse effects on sexual function and fertility

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 given in Table 33 below were appointed key studies by the RAC in its 2014 opinions on boric acid, disodium tetraborate 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 34 and 10.10.2.

Table 33: 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**
Boric acid			
Sub-chronic oral toxicity (90-day study) (<u>Study 1</u>	**For studies 1 and 2:**	**<u>Study 1 sub-chronic oral toxicity (rats):</u> 52.5 ppm boron (equivalent to 4.7 mg B/kg bw/day):**	Weir and Fisher 1972

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
and 2) Study 1: No guideline specified Rat (Sprague-Dawley) male/female n = 10/sex/dose group Study 2: 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 (Study 3)** No guideline specified, but conforms to the	Test material: boric acid or borax Purity: unknown Doses/conc.: -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 Exposure: 90 consecutive days prior to necropsy (daily in feed). **For study 3:** Test material: boric acid or borax Purity: unknown	One male and one female died during the study. Males: no changes in organ weights Females: 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):** Males: partial testes atrophy (5 rats) and spermatogenic arrest (1 rat). Females: 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. Males: 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. Females: 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). **Study 2 sub-chronic oral toxicity (dogs):** **17.5 ppm boron (equivalent to 0.4 mg B/kg bw/day):** Males: decreased spleen/body weight ratio (not specified if statistically significant, data not shown). Females: no reported changes in organ weights or organ/body weight ratios. **175 ppm boron (equivalent to 4.3 mg B/kg bw/day):** Males: decrease in testes/body weight ratio (not specified if statistically significant, data not shown). Females: no decrease in organ weight or organ/body weight ratios. **1750 ppm boron (equivalent to 43.7 mg B/kg bw/day):** One male dog died at day 68 of the study. Males: 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). Females: increased width of the zona glomerulosa of the adrenal glands; markedly atrophied thyroid glands with lymphoid tissue	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>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 groups were taken to the F2 and F3 generations.

Rat (Sprague-Dawley) male/female

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

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

Reliability: 2</td><td><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>: 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.</td><td>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="11">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="7">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.
<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:</td><td></td></tr>
</table>

<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>Exposure: 24 months, daily in feed.</td><td>
<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 (>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).</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		**At 28 days of exposure:** Over the 28-day study period, the rats consumed approx. 348.3 mg/kg bw/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 rating of cell types present, and a quantitative assessment of the number of late	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. Exposure: 27 weeks (daily in feed)	**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 ($p<0.05\%$) 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).	Fail et al. 1991

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
spermatids per testis was calculated as number of spermatids per gram of testis x 10^4. Reliability: 2		**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 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	Test material: 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.	**3000 ppm boric acid (equivalent to 26 mg B/kg 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.	Ku et al. 1993

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
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	Exposure: 9 weeks (daily in feed)	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: 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	Test material: boric acid	No information on general toxicity was available for any of the dose groups. **1 mg B/kg bw /day**	Marat et al. 2018

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
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 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	Purity: unknown 0, 1 and 10 mg B/kg bw/day Exposure: 60 days, daily oral gavage	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, $p \leq 0.05$).	

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
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 days exposure periods, 5 male rats from each dose group were serially mated with untreated female rats, in order to assess fertility.	Test material: Borax (disodium tetraborate decahydrate) Purity: unknown <u>Doses/conc.:</u> 0, 500, 1000 and 2000 ppm borax, equivalent to 0, 50, 100 and 200 mg B/kg bw/day, respectively. Exposure: 30 and 60	**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 (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%). <u>Serial mating</u>: 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	Lee et al. 1978

Method, guideline, deviations if any, species, strain, sex, no/group[4]	Test substance, dose levels duration of exposure	Results	Reference
Pregnancy rates were calculated as percentage of pregnant females/number of vaginal plugs. Reliability: 2	days (daily in diet)	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 bw/day.	

[4] Where applicable and unless stated otherwise, the reliability scores of the studies presented in Table 33 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 34: 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	The study did not observe statistical significant differences in sperm quality parameters (concentration,	Duydu et al., 2018a

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		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)	morphology, motility) or reproductive hormone levels (LSH, FH and testosterone) between exposure groups.	
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: 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	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. Furthermore, no significant effect observed on sex ratio at birth in groups exposed to boron vs. control group.	Duydu et al., 2019[5]
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	The mean blood boron concentration and mean semen boron concentration of the exposed group were significantly higher	Yalcin et al., 2019

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		Exposed group (n=86): 141.55 ng B/g blood	(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.	

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

Table 35: 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

No information from animal studies on adverse effects on sexual function and fertility of ulexite, colemanite and tincalconite is available.

Data on boric acid and borate salts

The assessment of adverse effects on sexual function and fertility of the borate minerals included in the present CLH proposal is based on 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 ulexite, colemanite and tincalconite and simple borates such as boric acid and borate salts will predominantly exist as undissociated boric acid. The toxicokinetic and toxicological properties of the borate minerals 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 publically disseminated REACH Registration dossier for boric acid), male and female rats were administered 0, 117, 350 and 1170 ppm boric acid (equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw/day, respectively). Seminiferous tubular degeneration and testicular atrophy was seen after 6, 12 and 24 months of treatment at 58.5 mg B/kg bw/day. At the end of treatment (24 months), the incidence of testicular atrophy was 10%, 40% and 100% at 5.9, 17.5 and 58.5 mg B/kg bw/day, respectively. Based on these findings, the NOAEL and LOAEL for rat fertility were 17.5 and 58.5 mg B/kg bw/day, respectively.

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.

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

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

At 26.6 mg B/kg bw/day, F0 male mice displayed significantly lower sperm motility than controls (by approx. 13%; $p<0.05$) in all 19/19 male mice, and no significant changes where revealed by the histopathological examination. The fertility index for the F0 generation was 100% for the first 4 litters and 84% for the fifth litter. No histopathological results were reported for female mice. The absolute body weights of males were comparable to controls (42.11 ± 1.16 vs. 42.24 ± 0.80 in controls). At 111.3 mg B/kg bw/day, statistically

significant ($p<0.05$) changes as compared to controls were seen in F0 male mice: decreased mean sperm concentration and mean percentage of motile sperm (by 72% and 32%, respectively), decreased seminiferous tubular diameter (by approx. 32%), increased mean percentage of abnormal sperm (61.17 ± 5.25 vs. 11.34 ± 0.91 in controls, i.e. by approx. 439%), decreased absolute testis, epididymis and prostate weight (by approx. 51%, 21% and 20%, respectively). The histopathology examination revealed degenerative changes in the majority of the tubules, unorganised layered epithelium germ cells and few mature spermatozoa (incidence not reported). The fertility index for the F0 parental generation from the mid-dose group decreased from 95% for the first litter to 85 %, 30% and 5% for the second, third, fourth and fifth litter, respectively. There were no significant changes in body weight, body weight gain or other signs of general toxicity observed in F0 male mice in this dose group. In F0 female mice, vaginal cytology revealed normal cyclicity and no changes on body weight or uterus weight were seen.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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 36. 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 36: 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.

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.

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.

Human data

No human data on adverse effects on sexual function and fertility of ulexite, colemanite and/or tincalconite is available.

Data on boron compunds

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 37 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 37).

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 37). 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 37). 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 37: 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 38). 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 38: 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 39 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 39: Comet assay results in sperm samples, lymphocytes and buccal cells according to the different exposure groups

Number of participants	**Mean tail intensity ± SD in sperm (alkaline comet assay) (%)**	**Mean tail intensity ± SD in sperm (neutral comet assay) (%)**	**Mean tail intensity ± SD in lymphocytes (comet assay) (%)**	**Mean micronucleus frequencies in buccal cells ± SD (micronucleus assay)**
Basaran et al. 2019 (Turkey)				
n = 12 (very low exposure)	5.37 ± 1.63 (3.1 – 8.42)	6.31 ± 1.16 (5.13 – 8.49)	6.0 ± 2.69 (2.82 – 11.95)	3.54 ± 2.73* (1 – 9)
n = 17 (low exposure)	5.61 ± 1.2 (3.97 – 8.96)	6.09 ± 1.1 (4.22 – 7.81)	7.79 ± 5.18 (1.85 – 24.5)	5.13 ± 4.69 (0 – 19)
n = 108 (medium exposure)	6.03 ± 4.83 (2.6 – 49.71)	6.23 ± 1.36 (3.95 – 13.68)	7.5 ± 5.34 (1.64 – 27.47)	4.32 ± 3.82 (0 – 19)
n = 50 (high exposure)	5.55 ± 1.88 (2.81 – 13.73)	6.16 ± 1.26 (4.12 – 9.66)	8.7 ± 7.94 (1.38 – 36.0)	4.56 ± 3.61 (0 – 16)
n = 25 (extreme exposure)	5.36 ± 1.88 (3.04 – 12.32)	5.71 ± 0.97 (4.24 – 8.4)	5.04 ± 2.26 (0.65 – 10.08)	4.06 ± 2.93* (0 – 10)

Correlations of blood B levels and genotoxicity parameters									
Correlations between blood B level and:	Sperm DNA damage	Lymphocyte DNA damage	MN	BN	CC	KHC	KYL	PYC	NBUD
Pearson correlations	0.028	-0.024	0.023	-0.052	-0.156*	0.047	-0.045	0.058	0.023

*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 40 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 40: 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

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 borax from animal studies showing adverse effects of boron on sexual function and fertility, fulfil the classification criteria for ulexite, colemanite and tincalconite 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 ulexite, colemanite and tincalconite were derived in a similar way by correcting for the percentage of boron (calculations are available in Table 49). These borate minerals 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 do not apply for ulexite, colemanite and tincalconite.

10.10.4 Adverse effects on development

With the exception of recent studies by Marat et al. 2018 and Pleus et al. 2018, the studies given in Table 41 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 42 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 41: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group[6]	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Boric acid and borax			
Prenatal Developmental Toxicity Study GLP-compliant Rat (Crl: CD VAF/Plus (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	Test material: boric acid Purity: 98% <u>Doses/conc.:</u> 0, 250, 500, 750, 1000, 2000 ppm boric acid equivalent to 0, 19, 36, 55, 76 and 143 mg boric acid/kg bw/day, respectively (equivalent to 0, 3.3, 6.3, 9.6, 13.3 and 25 mg B/kg bw/day) <u>Exposure phase I</u>: days 0 - 20 post mating (nominal in diet) <u>Exposure phase II</u>: days 0 - 20 post	**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 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.	Price et al. 1996a Price et al. 1997

Method, guideline, deviations if any, species, strain, sex, no/group[6]	Test substance, dose levels duration of exposure	Results	Reference
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.	mating (nominal in diet), then on normal diet until termination on day 21 postpartum	**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 and live foetuses were examined.	Test material: boric acid Purity: unknown Doses/conc.: 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 Exposure: treatment on days 6 - 19 post-mating, via oral gavage	**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, 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	Price et al. 1996b Heindel et al. 1994

Method, guideline, deviations if any, species, strain, sex, no/group[6]	Test substance, dose levels duration of exposure	Results	Reference
		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 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	**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. 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	Heindel et al. 1992

Method, guideline, deviations if any, species, strain, sex, no/group[6]	Test substance, dose levels duration of exposure	Results	Reference
	bw/day, equivalent to 0, 43, 79 and 175 mg B/kg bw/day, respectively Exposure (daily in feed): GD 0 – 17	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 bw/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 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)	
Reproductive toxicity assessment study No guideline	Test material: boric acid or borax Purity: unknown	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.	Weir and Fisher 1972

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group[6]</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>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>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>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="10">Live birth index[a]</td><td colspan="3">P1-F1A</td><td colspan="3">P1-F1B</td></tr>
<tr><td>98.4</td><td>98.4</td><td>100</td><td>99.1</td><td>99.2</td><td>99.4</td></tr>
<tr><td colspan="3">P2-F2A</td><td colspan="3">P2-F2B</td></tr>
<tr><td>97.8</td><td>99.4</td><td>96.9</td><td>98.6</td><td>92.4</td><td>98.8</td></tr>
<tr><td colspan="3">P3-F3A</td><td colspan="3">P3-F3B</td></tr>
<tr><td>100</td><td>100</td><td>99.4</td><td>100</td><td>100</td><td>100</td></tr>
<tr><td colspan="7">Boric acid</td></tr>
<tr><td colspan="3">P1-F1A</td><td colspan="3">P1-F1B</td></tr>
<tr><td>98.4</td><td>96</td><td>97.2</td><td>99.1</td><td>99.4</td><td>100</td></tr>
<tr><td colspan="3">P2-F2A</td><td colspan="3">P2-F2B</td></tr>
<tr><td>97.8</td><td>100</td><td>99.4</td><td>98.6</td><td>99.4</td><td>97.9</td></tr>
<tr><td colspan="3">P3-F3A</td><td colspan="3">P3-F3B</td></tr>
<tr><td>100</td><td>99.5</td><td>97.9</td><td>100</td><td>99</td><td>98.8</td></tr>
</table>
[a] Live birth index = number of pups born alive/number of born pups x 100.</td><td></td></tr>
<tr><td></td><td></td><td>Maternal effects</td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group[6]	Test substance, dose levels duration of exposure	Results	Reference
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 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	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/day, equivalent to 0, 26.6, 111.3 and 221 mg B/kg bw/day, respectively. Exposure: 27 weeks (daily in feed)	**9000 ppm** (equivalent to 221 mg B/kg/day): 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.	Fail et al. 1991
Assessment of embryonic or foetal death after treatment of male rats during spermatogenesis No guideline specified Rats (white outbred), male	Test material: boric acid Purity: unknown Doses/conc.: 0, 1 and 10 mg B/kg bw/day Exposure: 60 days, daily oral gavage	**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[6]	Test substance, dose levels duration of exposure	Results	Reference
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 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]

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

[6] Where applicable and unless stated otherwise, the reliability scores of the studies presented in Table 41 are according to the CLH dossier of boric acid, assessed by RAC in 2014.

Table 42: 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. This study is a follow-up of the same mother-child cohort as was investigated by Igra et al. 2016.	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 length.	Hjelm et al., 2019
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 was 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 43: 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

No information from animal studies on adverse effects on development of ulexite, colemanite and tincalconite is available.

Data on boric acid and borate salts

The assessment of adverse effects on the development of the offspring of the borate minerals included in the present CLH-proposal 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 ulexite, colemanite and tincalconite after oral exposure are therefore expected to be similar to boric acid and borate salts.

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

Price et al. 1996a conducted a GLP-compliant study where female rats were administered 0, 19, 36, 55, 76 and 143 mg boric acid (equivalent to 0, 3.3, 6.3, 9.6, 13.3, 25 mg B/kg bw, respectively) via diet in two different phases: Phase I when teratologic evaluation was performed (days 0 – 20 post-mating) and Phase II for postnatal evaluation (the dams delivered and the pups were sacrificed after weaning). No maternal deaths occurred and no treatment-related clinical signs of general toxicity were observed in the dams, at any dose level. A statistically significant reduction in the mean foetal body weight per litter was observed at the two highest dose levels (i.e. by approx. 6% at 13.3 mg B/kg bw/day and by approx. 13% at 25 mg B/kg bw/day, compared to controls). The viability of the offspring was not affected in any dose group. Treatment-related skeletal changes were observed at the highest dose levels. Thus, statistically significant increases in the incidence of short rib XIII (i.e. by approx. 1.5% at 13.3 mg B/kg bw/day and by approx. 3.4% at 25 mg B/kg bw/day, compared to controls) and wavy rib (by approx. 2.1 at 13.3 mg B/kg bw/day and by approx. 10% at 25 mg B/kg bw/day, compared to controls) amongst offspring were reported. Based on the observed results, the LOAEL for skeletal effects in rats was 13.3 mg B/kg bw/day and the NOAEL was 9.6 mg B/kg bw/day.

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

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

In two prenatal developmental toxicity studies, pregnant female rabbits were administered 0, 62.5, 125 and 250 mg/kg bw/day boric acid (equivalent to 0, 11, 22 and 44 mg B/kg bw/day) via oral gavage during GD 6 –

19. Increased incidence of vaginal bleeding, considered to be treatment-related (2 – 11 pregnant females/day bled between GD 19 – 30), was observed at the highest dose level 44 mg B/kg bw/day. All does with vaginal bleeding had no live foetuses on GD 30. Reduced food intake and body weight gain were reported at the highest dose level (statistically significantly reduced by approx. 31% and 10%, respectively, as compared to controls) during the treatment period. However, the corrected (for gravid uterus weight) maternal weight change was increased.

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

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

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

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

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

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

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

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

bw/day. The results of this study showed that rats had a greater sensitivity to the developmental effects of boric acid than mice.

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

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

Rodent dominant lethal test (Marat et al. 2018)

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

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:

1) Death of the developing organism

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

2) Structural abnormality

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

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

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

3) Altered growth

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

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

4) Functional deficiency

The CNS morphological defects (i.e. enlarged lateral ventricles of the brain) were seen in rats at 58 and 94 mg B/kg bw/day, and were considered to be developmental effects *per se* and not due to growth retardation

(Heindel et al. 1992). The implication of these neurodevelopmental effects on the functional development of rats is however not clear.

Human data

No human data of the borate minerals included in the present CLH-proposal on adverse effects on the development of the offspring is available.

Data on boron compunds

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 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 44, 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 ($r_s = 0.28$; $p = 0.0001$). 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 ($r_s = 0.82$). 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 ($r_s = 0.73$; $p < 0.001$) was stronger than the correlation with concentrations in maternal blood at GW 33 ($r_s = 0.41$; $p < 0.001$). 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; $r_s = 0.94$). The correlation with arsenic and lithium in breast milk was $r_s = 0.49$ 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 ($r_s > 0.1$).

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 44). 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 44: 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	Maternal serum	266 (47 – 624)

Perinatal exposure markers		Median (range) boron concentrations (µg/L)
Hjelm et al. 2019 (Argentina)		
(0 – 3 months after birth; n = 108)	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 45: 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)						
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

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
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 compunds

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

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 borax 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 ulexite, colemanite and tincalconite.

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 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 the borate minerals from the same LOAEL and by correcting for the percentage of boron (calculations are available in Table 49). Ulexite, colemanite and tincalconite 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.

10.10.7 Adverse effects on or via lactation

Table 46: 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**
Boric acid and borax (disodium tetraborate decahydrate)			
Reproductive toxicity assessment study No guideline specified, but conforms to the standard three-generation, 2 litters per	Test material: boric acid or borax Purity: unknown Doses/conc.: 0,	**Effects on or via lactation** Significantly higher ($p<0.05$) lactation indices were observed at 5.9 and 17.5 mg B/kg bw/day, for both boric acid and borax treatments, and at 17.5 mg B/kg bw/day, the P3-F3A generation administered borax showed a significantly ($p<0.05$) lower lactation index than controls (presented below).	Weir and Fisher 1972 Weir 1966

<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>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 (reliable with restrictions)</td><td>117, 350 and 1170 ppm boron, equivalent to 0, 5.9, 17.5 and 58.5 mg B/kg bw

<u>Exposure</u>: from the beginning of the study (14 weeks pre-mating exposure) until sacrifice of parents P1, and from weaning until sacrifice of the F1- and F2-generations (daily in feed).</td><td>
<table>
<tr><th>Index</th><th>Control</th><th>5.9 mg B/kg bw/day</th><th>17.5 mg B/kg bw/day</th><th>Control</th><th>5.9 mg B/kg bw/day</th><th>17.5 mg B/kg bw/day</th></tr>
<tr><td colspan="7">Borax</td></tr>
<tr><td rowspan="10">Lactation index[a]</td><td colspan="3">P1-F1A</td><td colspan="3">P1-F1B</td></tr>
<tr><td>56.3</td><td>63.6</td><td>82.3[b]</td><td>58.8</td><td>60</td><td>74.2</td></tr>
<tr><td colspan="3">P2-F2A</td><td colspan="3">P2-F2B</td></tr>
<tr><td>48.3</td><td>79.8b</td><td>82.7[b]</td><td>92.1</td><td>93.2</td><td>95.5</td></tr>
<tr><td colspan="3">P3-F3A</td><td colspan="3">P3-F3B</td></tr>
<tr><td>91.5</td><td>81.1</td><td>79.1[c]</td><td>89.7</td><td>91.8</td><td>95.9</td></tr>
<tr><td colspan="7">Boric acid</td></tr>
<tr><td colspan="3">P1-F1A</td><td colspan="3">P1-F1B</td></tr>
<tr><td>56.3</td><td>96.2</td><td>70.3[b]</td><td>58.8</td><td>85.6b</td><td>80[b]</td></tr>
<tr><td colspan="3">P2-F2A</td><td colspan="3">P2-F2B</td></tr>
<tr><td>48.3</td><td>79.2[b]</td><td>83.1[b]</td><td>92.1</td><td>81</td><td>98</td></tr>
<tr><td colspan="3">P3-F3A</td><td colspan="3">P3-F3B</td></tr>
<tr><td>91.5</td><td>82.5</td><td>86.5</td><td>89.7</td><td>86.7</td><td>87.9</td></tr>
</table>
[a] Lactation index = number of weaned pups/number left to nurse x 100.
[b] Significantly higher than controls.
[c] Significantly lower than controls.
</td><td></td></tr>
<tr><td>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</td><td>Test material: boric acid

Purity: >99%

<u>Doses/conc</u>.: 0, 1000 ppm, 4500 ppm or 9000 ppm equivalent to 0, 152, 636 and 1262 mg boric acid/kg bw/day, equivalent to 0,</td><td>Effects on or via lactation
During the lactation period, there were no effects on viability or growth of F1 or F2 pups at any dose level.</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
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 (reliable with restrictions)	26.6, 111.3 and 221 mg B/kg bw/day, respectively. Exposure: 27 weeks (daily in feed)		
Prenatal Developmental Toxicity Study GLP-compliant Rat (Crl: CD VAF/Plus (Sprague Dawley)) n = groups of 14 – 17 females/dose group/phase Reliability: 1 (reliable without restriction), key study In phase II the dams were allowed to deliver and the pups reared to weaning and then killed for full visceral and skeletal examination.	Test material: boric acid Purity: 98% Doses/conc.: 0, 250, 500, 750, 1000, 2000 ppm boric acid equivalent to 0, 19, 36, 55, 76 and 143 mg boric acid/kg bw/day, respectively (equivalent to 0, 3.3, 6.3, 9.6, 13.3 and 25 mg B/kg bw/day) Exposure phase II: days 0 - 20 post mating (nominal in diet), then on normal diet until termination on PND 21	**Effects on or via lactation** During lactation and until PND 21, there were no effects on viability or growth of the offspring at any dose level.	Price et al. 1996a

Table 47: 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 48: 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 studies

No information from animal studies on the effects of ulexite, colemanite and tincalconite on or *via* lactation is available. Since these borate minerals 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 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 ulexite, colemanite and tincalconite on or *via* lactation was available. Since these borate minerals 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 statistical 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 ulexite, colemanite or tincalconite, 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 ulexite, colemanite and tincalconite for adverse effects on sexual function and fertility; and adverse effects on development of the offspring is warranted: **Repr. 1B, H360 FD**.

Classification of these borate minerals 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 49: Derivation of ED10 values and concentration limits for ulexite, colemanite and tincalconite based on boron content

Substance	Molecular formula	Molecular weight (g/mol)	Conversion factor for equivalent	ED10 for fertility corrected for boron-content (mg/kg bw/day)**	LOAEL for development corrected for	Proposed generic concentration limit (GCL,	Proposed generic concentration limit (GCL,

			dose of boron*		boron-content (mg/kg bw/day)***	% w/w), fertility	% w/w), development
Ulexite	$B_5CaH_{16}NaO_{17}$	405.3	0.13	17.5/0.13=134.6	13.3/0.13=102.3	0.3	0.3
Colemanite	$B_6Ca_2H_{10}O_{16}$	411.1	0.16	17.5/0.16=109.4	13.3/0.16=83.1	0.3	0.3
Tincalconite	$B_4H_{10}Na_2O_{12}$	291.3	0.15	17.5/0.15=116.7	13.3/0.15=88.7	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 assessed in this CLH-proposal.

10.12 Specific target organ toxicity-repeated exposure

Not assessed in this CLH-proposal.

10.13 Aspiration hazard

Not assessed in this CLH-proposal.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed in this CLH-proposal.

12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this CLH-proposal.

13 ADDITIONAL LABELLING

Not relevant.

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15 ANNEXES

No annexes